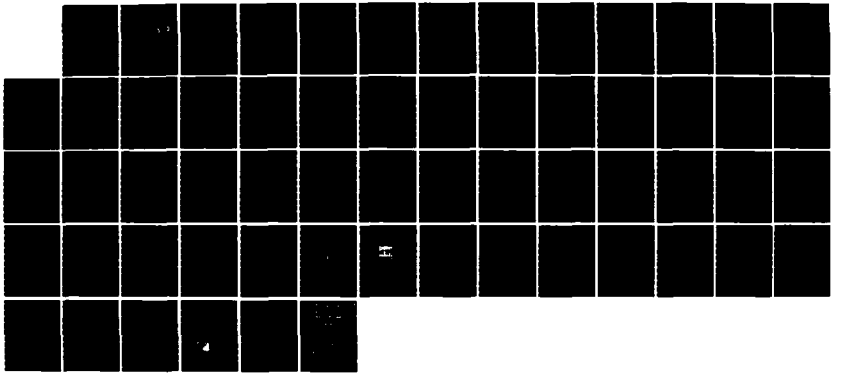
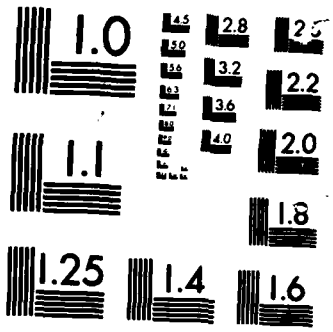
AD-A166 435 TRYPANOSOME SURFACE ANTIGEN GENES: ANALYSIS USING RECOMBINANT DNA(U) ISSAQUAH HEALTH RESEARCH INST WA K D STUART 15 JUN 84 DAMD17-82-C-2016 1/1

UNCLASSIFIED F/G 6/13 NL





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TRYPANOSOME SURFACE ANTIGEN GENES:

ANALYSIS USING RECOMBINANT DNA

ANNUAL REPORT

12/2/82 - 12/1/83

KENNETH D. STUART, Ph.D.

June 15, 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701-5012.pa

Contract No. DAMD17-82-C-2016

Issaquah Health Research Institute
1595 N.W. Gilman Blvd.
Issaquaqh, Washington 98027

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Issaquah Health Research Institute 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 1595 N.W. Gilman Boulevard Issaquah, Washington 98027	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-82-C-2016
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3M162770A870; TASK NO. AH; WORK UNIT ACCESSION NO. 008

11. TITLE (Include Security Classification)
Trypanosome Surface Antigen Genes: Analysis Using Recombinant DNA.

12. PERSONAL AUTHOR(S)
Kenneth D. Stuart, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 82/12/2 TO 83/12/1	1984 June 15	58

16. SUPPLEMENTARY NOTATION

Cont'd

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	
06	13	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
African Trypanosomes, Surface antigens, Sleeping sickness, Gene cloning.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The objective of this project is to identify and isolate early (frequently expressed) variant antigenic antigenic types (VATs), and clone and characterize their expressed variant surface glycoprotein (VSG) genes. Numerous syringe passaged and cyclically transmitted, frequently expressed VATs have been isolated, monoclonal antibodies prepared to their VSGs, and the expressed VSG genes have been cloned. We have shown that many diverse stocks express VSG epitopes related to the early IsTat epitopes. The VSG gene organization in the genome and sequence organization has been characterized. We have confirmed sequence homology at the 3' terminus of the VSG genes and have discovered additional homology near the 5' terminus of unrelated VSG genes. Numerous relapse VATs have also been isolated and characterized with respect to telomeric location of VSG genes and duplicative or non-duplicative mode of VSG gene expression. We have found that two

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia Miller	22b. TELEPHONE (Include Area Code) 301/663-7325 22c. OFFICE SYMBOL SGRD-RMS

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

fundamental processes control the expression of VSG genes. One involves gene duplication; the other telomeric activation. In addition, the telomeric location of the gene affects its probability of expression and stability. DNA sequence analysis in and around flanking VSG genes has characterized some of the internal homology and identified flanking sequence homology blocks. The latter probably function in duplicative activation of VSG genes. Keywords:

See DD1473

12/2/82 TO 12/1/83

SUMMARY

PURPOSE

The purpose of this study is to identify, isolate and characterize the variant surface glycoprotein (VSG) genes that are expressed by frequently occurring variant antigenic types (VATs) of African trypanosomes. This study will investigate if the coding and flanking sequences of VSG genes that encode different VSGs have nucleotide sequence characteristics in common. It will also be determined if various stocks of African trypanosomes frequently express VSGs related to those being studied.

METHODS

Six VATs were selected for detailed analysis based on previous work that produced this well characterized VATs, monoclonal antibodies specific for the VSGs expressed by these VATs, cDNA clones corresponding to the mRNA of the expressed VSG genes and genomic clones of members of the VSG genes families under study. The order of VAT expression and VSGs expressed among stocks were performed using mouse model systems and indirect immunofluorescences with the monoclonal antibodies. The monoclonal antibodies were studied in detail by a variety of standard immunological and biochemical techniques to determine their specificity. The genomic organization of the VSG genes was examined using Southern blotting analysis combined with mapping and nuclease studies. The cDNA and genomic clones were examined in detail by nucleotide sequence analysis and transcripts were examined by Northern and primer extension analysis. The predominantly expressed VSG gene was identified from multiple relapses and examined similar to the cDNA and gDNA described above.

RESULTS AND CONCLUSIONS

A characteristic sequence of VAT production and the predominant VAT in the IsTaR 1 serodeme was identified. These were similar to those isolated from the original infection. Spontaneous relapse studies showed that growth rate and switching frequency affect the order of VAT occurrance. The early IsTaR 1 VATs are expressed frequently in primary and first relapse populations in a variety of stocks. Thus the early VSG gene sequences are conserved and frequently expressed. The 55 VSG specific monoclonal antibodies appear to each recognize distinct epitopes although some of these recognize epitopes that are near each other. Each VSG cDNA was mapped in detail and cross hybridization and sequence analysis revealed conserved sequences adjacent to the 3′ end of the coding sequence and near the 5′ end of the coding sequence. Each VSG gene was found to be telomeric when expressed. One VSG gene cloned as a cDNA was examined in detail and was expressed in the bacterial host. All VSG mRNAs had the same 35 nucleotide sequence on the 5′ terminus. This sequence

appears to be encoded at a site unlinked to the coding sequence and added posttranscriptionally. This appears to be a novel mechanism. Many of the VSG gene family members conserve a repeated sequence 5′ to the coding sequence. This may function in gene conversion to mediate antigenic variation. The presence of the additional VSG genes created by gene conversion is insufficient to activate the gene since inactive genes of this type occur following switching to an alternate VAT or conversion to procyclic forms. Other genes that are already telomeric may also be activated by a different mechanism.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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(1). OBJECTIVES.

Our integrated biological/immunological/molecular genetic approach to the analysis of antigenic variation will be continued. This approach has permitted us to recognize features of antigenic variation that are characteristic of early and predominant VATs and to detect new molecular events associated with antigenic variation.

(1.1) Total Program Objectives

The long term objective of the total program is to determine the molecular basis for antigenic variation. As indicated in the background section, the molecular mechanisms must account for several characteristics of antigenic variation in addition to the control of VSG gene expression. The more basic aspects of the program are being conducted with NIH support. These include the isolation and characterization of VSG genes and especially their flanking sequences. These studies are directed at further elucidation of genomic rearrangements associated with antigenic variation and the mechanisms regulating VSG gene expression.

(1.2) Total Project Objectives

The objectives of the project supported by the USAMRDC focus on questions that are less basic in nature and more supportive of USAMRDC programs. The overall objective of the project supported by the USAMRDC is to determine if there exist restricted numbers of VSG genes or gene segments that encode VSGs or VSG segments that are expressed early in the natural infection. The presence and early expression of these genes or gene segments in natural populations and various species of African trypanosomes, especially human pathogens, will be tested.

(1.3) The objectives for the period for which support is requested are:

1. To identify, isolate and characterize VATs which initiate or occur early in infections.

This objective is designed to determine if a clone expresses the same VATs in metacyclic, predominant and early VAT populations and if the ame VATs remain metacyclic, predominant and early VATs following multiple rounds of cyclic transmission. We found that syringe passaged clones express the same predominant and early VATs, but derived cyclically transmitted clones did not. We wish to confirm these observations and extend them to metacyclic forms.

2. To determine if predominant, early and metacyclic VSGs and their corresponding coding DNA sequences have common characteristics.

These studies are designed to detect potential immunological

cross reaction among predominant, early and metacyclic VSGs and DNA sequence characteristics shared by the corresponding VSG genes. We will continue and extend the comparisons among predominant, early and meta- cyclic VSG genes that are in progress and compare these results with similar studies of later VSG genes.

3. To characterize predominant, early and metacyclic VAT VSG gene and flanking sequences.

These experiments are designed to determine the molecular basis for the frequent expression of predominant, early and metacyclic VSG genes. Our studies suggest that a telomeric location of basic copy VSG genes is the basis for their frequent expression. We will extend these studies to metacyclic VSG genes and examine in more detail the molecular basis for this more frequent expression.

4. To determine if the IsTaR I predominant, early and metacyclic VATs occur as frequently expressed VATs in diverse stocks of trypanosomes, especially human pathogens.

These studies are designed to extend our observations that many stocks express the IsTaR 1 VATs to determine their frequency of expression, and if regions of VSG homology are shared among these stocks. These studies are designed to assess the feasibility of common epitope based vaccines.

Sequence of Experiments:

The priorities for performing experiments are indicated at several points in the methods section. In general, the experiments of objective 1 will be performed over much of the grant period although these will require a small fraction of effort at any time. This is a result of biological factors affecting the rate at which these experiments can be done. We plan to complete most of objective 2 in the 1983-84 grant year, especially the analysis of VSG gene coding sequences. The genomic organization studies will extend into the 1984-85 year, as will the analysis of the metacyclic VSG gene coding sequences and their genomic organization. The experiments in objective 3 utilizing existing clones will be initiated early in the grant period as will related cloning and subcloning experiments. The analyses of the clones generated will be performed in the 1984/85 contract year. The experiments described in objective 4 will be done in the 1984-85 contract year.

(2.) PROGRESS REPORT.

This progress report covers the period of December 1, 1982 to December 1, 1983. A small fraction of the data presented in this report was the result of work supported by NIH. It is included for the sake of clarity and is identified where necessary.

During the current period of support we have concentrated our efforts on the syringe passaged IsTat 1 VATs 1.A, 1.1, 1.3, 1.5, 1.7 and 1.11 (A, 1, 3, 5, 7, and 11 henceforth) since the cellular material, antisera, monoclonal antibodies, cDNA and gDNA clones and recombinant libraries for these VATs were available. However, we have used these reagents and probes to examine other stocks and species of trypansomes as described below. The production of the IsTaR 1 serodeme, the initial biochemical characterization of the syringe passaged VATs and the initial molecular characterization of the VSG genes are described in publications. We have established a collaboration with Dr. K. Esser (Walter Reed Army Institute of Research) to secure metacyclic VATs and anti-metacyclic VAT antibodies and to accomplish cyclic transmission of specific VATs.

BIOLOGICAL (2.1) VAT Sequence

The A, 1, 3, 5, 7, and 11 VATs are early syringe passaged VATs y the criterion that they repeatedly occur within the first 45 days in rats, mice or ^&Peromyscus\& infected with any syringe passaged VAT of the IsTaR 1 serodeme. In addition, these VATs occur in the same sequence in all of these infections. A precedes all other VATs and the order of occurrence is conserved.

Figure 1 diagrams the first detectable occurrence of these VATs in several infections. A precedes all other VATs and the order of occurrence is compatible with the initial isolation. These data do not require that an obligate sequence of VAT production of A to 1 to 3 to 5 to 7 then to 11. Our data (see below) suggest a stochastic model for VAT switching with each VAT having a characteristic generation time. Thus, the occurrence of VATs in the bloodstream would reflect the switch probability, generation time and other factors such as immunogenicity and tissue tropism.

While the sequence of VAT occurrence was predictable in the syringe passaged line it was not in the cyclically transmitted line. Thus, it appears that the characteristic of being early or predominant VATs is not conserved through this cyclic transmission. VAT A is the most predominant VAT of the IsTaR 1 syringe passaged serodeme. This VAT invariably occurs in the first relapse of infections initiated with any IsTat 1 syringe passaged VAT and comprises essentially 100% of the relapse population. (Examples Fig.#1A and 1C). First relapse populations for VAT A are usually about 70% VAT 1 plus other VAT(s) or which we have no antisera (data not shown).

The relapse to A from the other VATs occurs spontaneously since as shown in Fig.#2 they occur in lethally irradiated animals. The VATs differ in the time from infection required for the population to convert to being predominantly VAT A (compare 2B, C and F). VAT 3 converts to A in about 20 days while VAT 1 takes about 50 days and VAT 5 is very variable taking as few as 5 days or as many as 20 depending on the experiment see Table 1. In order to analyze these results we constructed theoretical curves

(Fig.#3) to examine the proportion of the populations that would be VAT A with differing frequencies of VAT switching to A and differing growth rates of the injected VATs. It is clear from this set of curves that the time when the population changes to A (X) depends on the frequency at which the VATs in the population switch to A while the rate of population switching to A (X) (i.e. slope of % of A curve) depends on the growth rate of A relative to the previous VAT. Since all of the VATs switched to A and A was observed at a low level in all the syringe passaged populations (see 2B) then the VATs switch to A more frequently than any other VAT.

Modest differences in the relative generation times had large effects on the rate of switching to A. However, it was not possible to measure the growth rates of the VATs with sufficient accuracy to compare them with that of VAT A (Table 1). Therefore we constructed populations that were 50% A and 50% of another VAT and observed their relative proportions in the population Fig.#4 paralleled the spontaneous switch experiments (Table 1). Those populations that converted to A most quickly were "overgrown" by A while those which slowly switched to A were not overgrown by A.

From these results it is evident that A is the most predominant IsTaR 1 VAT because it is switched to most frequently and grows more rapidly than other VATs. In animals immune to A (because of relapse to A) other VATs are switched to at different frequencies and grow at different rates. These and other factors may be responsible for the sequence of VAT occurrence in the bloodstream. Data bearing on the molecular basis for predominance, or why VATs switch to A at higher frequency are presented below. In addition, it appears that in our original collection of VATs 7 may be in the same lineage as 3 but not intervened by 5. This conclusion is based on the detection of an extra copy of the 3 VSG gene in 3 and 7, but not 5 as presented below.

(2.2) Cyclic transmission/IsTaR 1 VATs in Other Stocks

Relapses from cyclically transmitted populations derived from the IsTaR 1 serodeme do not relapse to VAT A. Similarly, the original stock from which the IsTaR 1 serodeme was derived after laboratory maintenance and clones from his original stock do not relapse to A. Immunofluorescence analysis with monoclonal antibodies of relapse populations taken at four day intervals over 60 day infections of Peromyscus that were initiated with the cyclically transmitted line revealed no early IsTaR 1 VATs. VAT A was recovered from the 60 day population after cloning but this may have been the result of a relapse. Thus, while VAT A is the predominant VAT for the syringe passaged IsTaR 1 serodeme, it does not remain so after transmission and was not in the original field isolate or in clones derived from the field isolate without laboratory maintainance (syringe passage). This observation requires repeating and coordinated molecular genetic analysis.

Monoclonal antibody cocktails were used to examine 22 stocks

of trypanosomes representing Trypanosoma brucei, gambiense, rhodesiense, equinum and evansi. Monoclonals were used to detect expressed antigens rather than related gene sequences. The later studies are being done in collaboration with Dr.#Agabian (U.#Washington). The stocks varied with respect to host (human, animal, tsetse fly), geographical origin, length and type of laboratory maintenance, cyclic transmission and whether cloned. First parasitemia and first relapse populations were examined. As seen in Table 2, 18 of 22 stocks expressed VSGs that reacted with early IsTaR 1 anti- VSG monoclonal antibodies. Most of these stocks expressed several VSGs related to the early IsTaR 1 VSGs. Those stocks that did not express these VSGs tended to be cloned stocks whose relapses were not examined. In addition, no evansi stocks expressed the early IsTaR 1 VSGs. We, therefore, conclude that many African trypanosome stocks may express VSGs that are related to early IsTaR 1 VSGs. It is striking that these VSGs were observed so frequently early in the infection in populations of the other stocks.

IMMUNOLOGY (2.3) Monoclonal Antibodies

Monoclonal antibodies have been prepared against VSGs purified from the early IsTat 1 VATs. These studies are detailed in publications. Briefly, 55 of the 82 McAbs studied were specific for VSGs and each McAb appears to be specific for a distinct epitope. Table 3 summarizes the characteristics of these VSG specific McAbs. All of these McAbs give a VAT specific reaction with isolated VSG or total cell lysates in an ELIZA assay and with acetone fixed cells in an indirect immunofluorescence assay. The one exception is that one McAb reacted with VSG from 11 and from 1 (which is indistinguishable from VSG D). This is probably a spurious cross reaction given the lower affinity of the reaction. Seventeen McAbs reacted with live cells in indirect immunofluorescence assays but only one of these reacted with SDS treated VSG in western blots indicating that most epitopes exposed on the surface include a secondary structural component. The monoclonal antibodies that did not react with live cells by IFA reacted with SDS treated VSG and with in vitro translated antigen suggesting that the epitopes recognized by these antibodies rely on the primary structure of the antigen. All of the above antibodies were VAT and VSG specific IgGs. However, 27 other antibodies were not VAT specific and about half of these were IgMs suggesting that they were originally selected during screening on a basis which reflects the lack of specificity of many observed for IgMs. Several possibilities concerning the specificity of these antibodies exist, including trivial explanations such as some are directed against contaminants. However, we have not excluded the possibility that at least some of the antibodies are directed against VSG epitopes that occur on various VSGs but do not react with the antibodies in unfixed or acetone fixed cells as described in the accompanying paper.

MOLECULAR GENETIC (2.4) VSG Genes

(2.4.1) Expressed VSG Genes

Each of the A, 1 (D), 3, 5, 7, and 11 expressed VSG genes has been cloned as a series of cDNA clones. Some of these cDNAs and their maps are shown in Figure 5. Each of the expressed VSG genes has also been restriction mapped by a combination of mapping the cDNA clones and mapping genomic DNA using the cDNA clones as probes. Composite maps of the expressed VSG genes are presented in Figure 6. A few landmarks are indicated in Fig.#6A. The maps 5′ to the barren region have limited accuracy due to technical limitations and some sites cannot be mapped if a site for that enzyme occurs immediately 5′ to the gene as is quite common. Nevertheless, the maps 5′ to the telomeres which contain VSG genes are similar. This will be described in more detail below when the VAT A genes are discussed.

Each expressed VSG gene resides adjacent to a telomere (end of a chromosome) and is flanked by barren region DNA which is devoid of restriction sites (see 6A). The telomeric location of these genes has been demonstrated by BAL 31 sensitivity, (data not shown) telomeric growth and presence of a DNA end detected as an apparent restriction site for all enzymes tested (see 6C). The presence of these barren regions has complicated both the mapping and genomic cloning of these genes. The telomere grows by nucleotide addition as illustrated in Figure 7 and is periodically reversed by a large deletion. The 1.3 VSG gene DNA fragment indicated by the arrow increases in size in each VAT proceeding from A to 7. This is due to telomeric growth. The reduction in the size of this fragment in VAT 11 is the result of a substantial deletion of telomeric DNA. This telomere, to the extent that it has been mapped, is identical to the expressed 1.3 gene (ELC) which is present as an additional fragment in VAT 1.3 DNA. The additional fragment present in VAT 1.7 DNA is the lingering 1.3 ELC (L-ELC) and also has a map indistinguishable from the ELC (and telomeric gene). This suggests that VAT 5 is not a VAT that intervened in sequence between VATs 3 and 7 and VAT 7 is in a direct cell lineage from VAT 3. Telomeric growth does not appear to be related to VAT switching since it occurs on telomeres containing either expressed or non-expressed VSG genes and upon growth of populations without VAT switching (data not shown). The possibility that the telomeric deletion may be associated with VAT switching has not yet been excluded.

Much of the expressed 11 gene including its 5′ and 3′ ends, the 3′ end of the expressed 1 gene and a central segment of the expressed A genes have been sequenced from cDNAs (Fig.#8). With N.I.H. support and in collaboration with Dr. Agabian, we have shown one of these cDNAs to express antigen in E. coli. This is detailed in publication 7. The 5′ end of the 11 gene is recognized from the 35 nucleotide sequence (35-mer) and the 3′ ends of the 11 and 1 genes are identified from their poly (A) tails.

Both the 11 and 1 gene 3′ sequences translate into a C-terminal hydrophobic amino acid sequence that resembles that of

other VSGs (like ILTat 1.3 and 221), (Figure 9). The more N terminal segment of this region contains the conserved ser and lys and the tripeptide adjacent to the termination codon is conserved to the same extent as in other VSGs (it is not always leu, leu, phe). In addition, many other amino acids are conserved. The 3′ untranslated 11 sequence has a great deal of homology (about 75%) to other VSG genes, while the 3′ untranslated sequence from 1 does not. The 11 gene contains two CCTC, an AmTn, an octomer and a 14-mer sequence that occur in other VSG genes (like 221). In contrast, the 1 gene contains a short untranslated 3′ tail. This sequence lacks the CCTC and AmTn elements but it does contain 7 of the 8 octomer nucleotides and perhaps a fragment of the 14 mer sequence. In addition its five 3′ terminal nucleotides match five subterminal nucleotides of 11. While these results are preliminary and require sequencing of other VAT 1 cDNAs to avoid cloning artifact, they suggest that the early VSG gene differs substantially from the later VAT 11 gene. This difference may reflect the NDA mechanism of VAT 1 gene expression rather than its early expression. The molecular basis for early versus later is discussed below. The NDA mechanism of expression probably does not employ the 3′ homology blocks represented in the untranslated 3′ end in VAT 11.

The 5′ region which would be common and unique to all expressed VSG genes and would be expected to contain the promoter has not been localized by restriction mapping. It does not occur within about 50 kb 5′ to the expressed gene. This has also been observed in other labs. With NIH support and in collaboration with Dr.Agabian, a 35 nucleotide sequence found on the 5′ end of VSG mRNAs has been found to occur in a large tandem repeated cluster or clusters in the genome and also occurs in orphons. This work is detailed in publications 8 and 9. The presence of one orphon is correlated with the presence of an ELC. Interestingly, we have recently observed that telomeric DNA containing ELCs or residual ELCs (ELCs remaining following a VAT switch) are hypersensitive to Bal 31. This implies that DNA from expressed or recently expressed telomeres differs from other telomeres.

The number of VSG genes differs among the different VATs and some, but not all, of these genes have a telomeric location. Of the VATs that we have studied all have duplications and transpositions of the a VSG gene in the expressor (an expression linked copy-ELC) except for VAT 1 which uses the NDA mechanism. The number of genes varies from 1 in VAT 1 to about 10 in VAT 3. These data are summarized in Table 4. All the ELCs have a telomeric location but non-expressed VSG genes also can have a telomeric location. For example, the VAT 1 gene is telomeric whether or not expressed. VATs A, 1, 3 and 5 have a non-expressed telomeric A, 1, 3 and 5 VSG gene, respectively both in expressors and non-expressors. VATs A, 3 and 5 produce an ELC upon expression while VAT 1 does not. VATs 7 and 11 have no 7 and 11 telomeric 7 and 11 genes except the ELCs produced in expressors. These data are presented in detail in publications 3 and 10. This observation and the detailed analysis of VAT A genes

suggests that a telomeric location of a VSG gene is the basis of predominance and early expression. Metacyclic VATs VSG genes may also have a characteristic chromosomal location.

(2.4.2) Genomic Clones

Numerous genomic DNA clones representing two VSG genes from VAT 3, two genes from VAT 5 and one gene from VAT A have been isolated using the lambda 1059 vector. All of these clones have been restriction mapped and are derived from the non telomeric genes. The A gene clone may represent a basic copy gene but the 3 and 5 gene clones do not represent basic copies of the genes cloned as cDNAs. Despite intensive efforts the 1 gene was not found in the 1 lambda library. This probably reflects the difficulty in cloning telomeric genes in general and particularly telomeric genes flanked by barren regions, using conventional means. Maps of the cloned genomic segments and the positions of the genomic clones are presented in Figure 10. Many segments of these genomic clones have been subcloned into plasmid vectors for detailed analysis. In summary, as indicated in Table 4, the single non telomeric A gene has been cloned while the expressed telomeric gene has been cloned as cDNAs. These may represent all the A genes since the two telomeric A genes may be identical (see below). The single 1 gene has been cloned as cDNAs. Both non telomeric 5 genes have been cloned and the expressed gene has been cloned as cDNAs. These represent all the 5 genes - the 2 non telomeric genes cloned as genomic clones and the expressed 3 gene as cDNAs representing 3 of the 8 to 10 VAT 3 genes. VAT 7 and 11 genomic clones have been isolated but have not yet been mapped or analyzed.

The genomic clones have been probed with a 70 bp repeat sequence which occurs 5′ to the ELC and BC. As indicated in Figure 10, this sequence has been mapped immediately 5′ to the A gene (10A). It also maps 5′ to one of the 3 genes and maps 3′ to the same gene (10C). This second site of hybridization probably indicates the position of another VSG gene. However, the 70 bp repeat sequence does not occur in the clones containing the two 5 genes nor one of the 3 genes (10B,D,E). Thus the 70 bp sequence is not adjacent to all VSG genes. We have also probed with a synthetic oligonucleotides representing the 5′ cDNA terminal sequence (35-mer) and the 3′ cDNA terminal sequence (14-mer). These sequences do not occur in any of the genomic clones.

A cDNA which contains about 450 3′ terminal nucleotides of the 1 VSG gene and the poly A tail hybridized to the genomic clones as indicated in Figure 10B,C,D. This hybridization was eliminated by addition of poly A indicating that the hybridization may have reflected the presence of the poly A tail or oligo T (A) stretches in the probe. The significance of this hybridization is as yet unknown but could represent integration of a sequence by the reverse transcription mechanism.

(2.4.3) Predominant VSG Gene

The A gene has been examined and mapped in detail since A is the most predominant VAT and relapses to this VAT from the other early IsTat VATs were available. The results of this study are presented in detail in a publication. Briefly, of the 6 independant A VATs that have been examined, two have ELCs while four do not. Hence, the important and surprising result that VAT A can be expressed by both the ELC and NDA mechanisms. Figure 11 shows a Southern blot revealing the ELC in two A VATs (Ae and A7) and absent in the others. As shown in Figure 12, NDA type VAT A expressors and a VAT 1 expressor have two A VSG genes; one telomeric and one non telomeric while ELC type VAT A expressors have two telomeric and one non telomeric genes.

The restriction maps of the coding sequences of all these A genes are identical. In addition, the maps of the telomeric A genes are identical except for the size of the 5′ barren region and telomere length. The map of the non telomeric A gene diverges from that of the telomeric A gene on both the 5′ and 3′ flanks. DNase sensitivity studies show that only one of the telomeric A genes in the ELC type of A VATs is expressed. (Figure 13).

These studies do not reveal the origin (basic copy) of the A ELC. If it arose from the non telomeric A gene then an unusually small segment would have been duplicated and transposed. A further unusual feature is that the two A ELCs that would have resulted from this duplication/ transposition are identical around the tranposition site (12b,c,f,g). Although this is conceivable, it is difficult to reconcile with the altered 5′ barren region that is detected as a Rsa I site closer to the gene (12c). It is therefore more likely that the telomeric gene was the origin of the A ELCs. The restriction maps of the ELCs and telomeric genes are identical except well 5′ to the gene and the length of the telomere. Based on these data, we propose that the A ELCs arose by a novel mechanism which we term telomeric gene conversion. By this mechanism, a strand from the A telomere would invade the expressed VSG gene telomere well 5′ to the gene and convert this invaded telomere to an A VSG gene containing region.

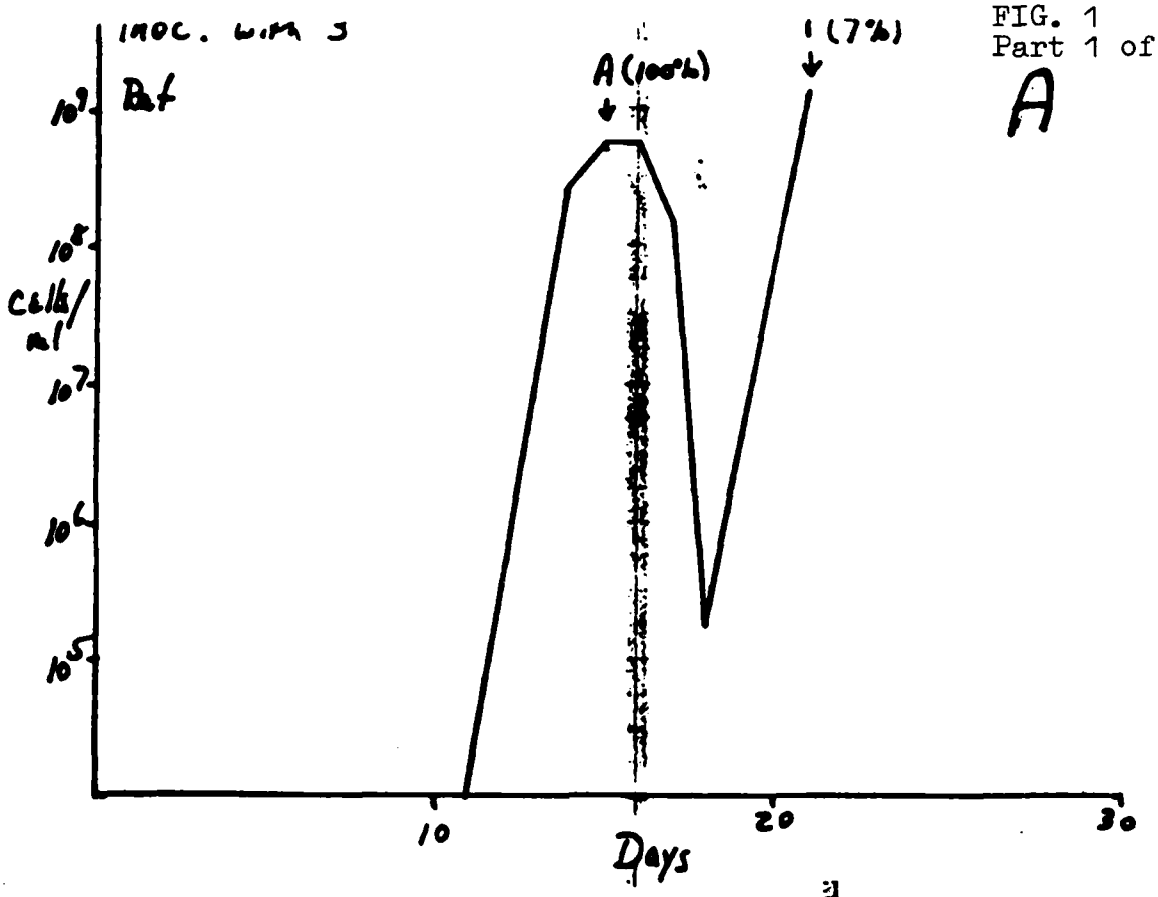
The ability of the A VSG gene to be expressed by both the ELC and NDA (and perhaps telomeric conversion) mechanisms may be the basis for the predominance of this VAT. As mentioned previously, non expressed 1, 3, and 5 VSG genes occur on telomeres. These early VATs are expressed by the NDA mechanism in VAT 1 and by the ELC or telomeric gene conversion in VATs 3 and 5. Thus VATs 1, 3 and 5 may be early VATs as a result of the telomeric location of these VSG genes. The 7 and 11 non expressed VSG genes are not located on telomeres in these later VATs. These VATs are expressed only by the ELC mechanism. Thus, later expression may be the result of non telomeric location of the basic copy genes.

Like the A gene, the restriction map of the 3 ELC is identical to that of the other telomeric 3 gene in 3 VATs.

However, the telomeric 1.5 gene map differs from the 1.5 ELC. (Fig. 6D, E).

While it had been originally reported by others that procyclic forms lost the ELC, thus explaining cessation of VSG gene activity, we found this not to be the case as detailed in publications 11 and 12. All VATs which expressed the VSG gene by the ELC mechanism retained this ELC when converted to procyclic forms.

FIG. 1
Part 1 of 2



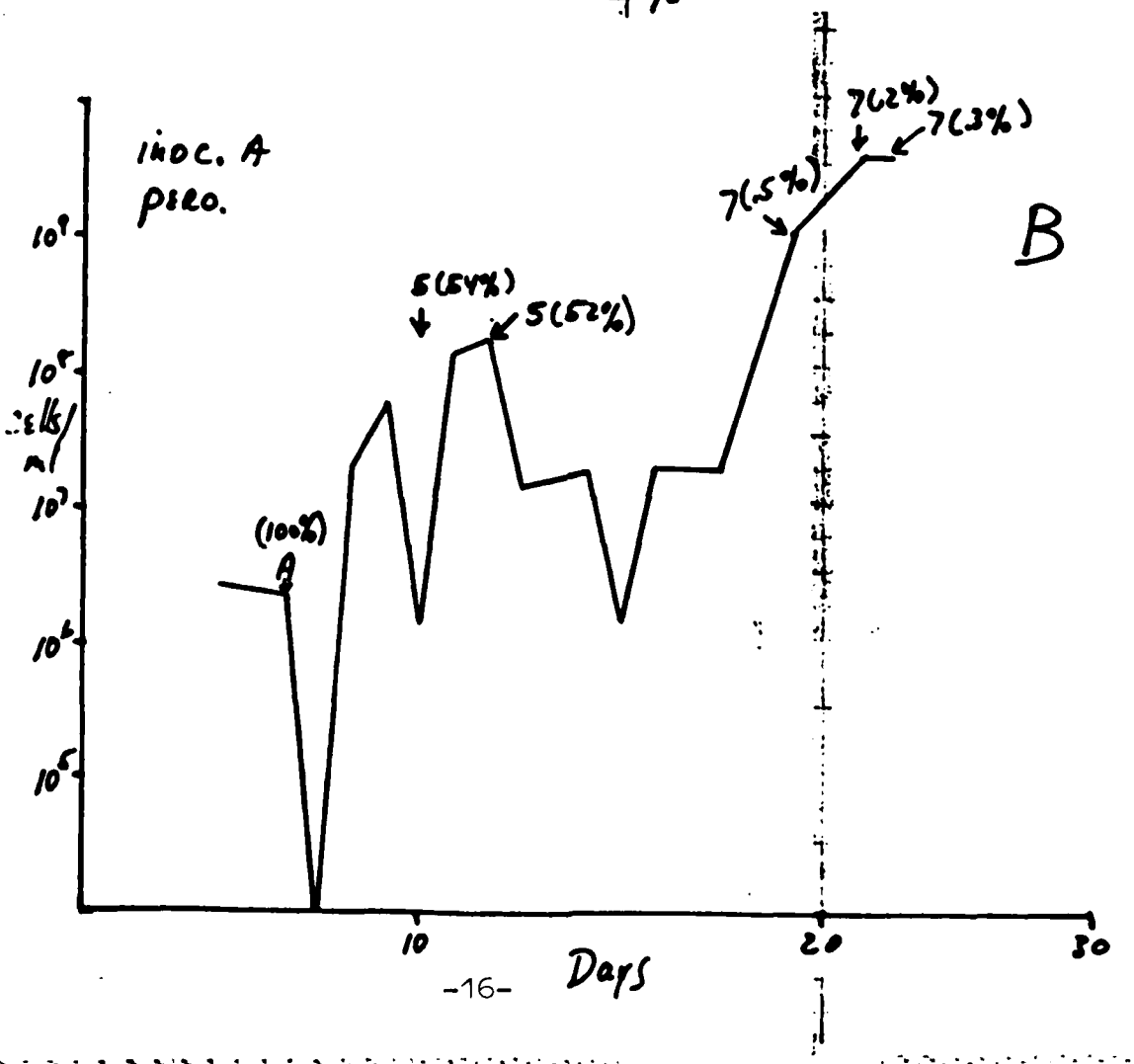
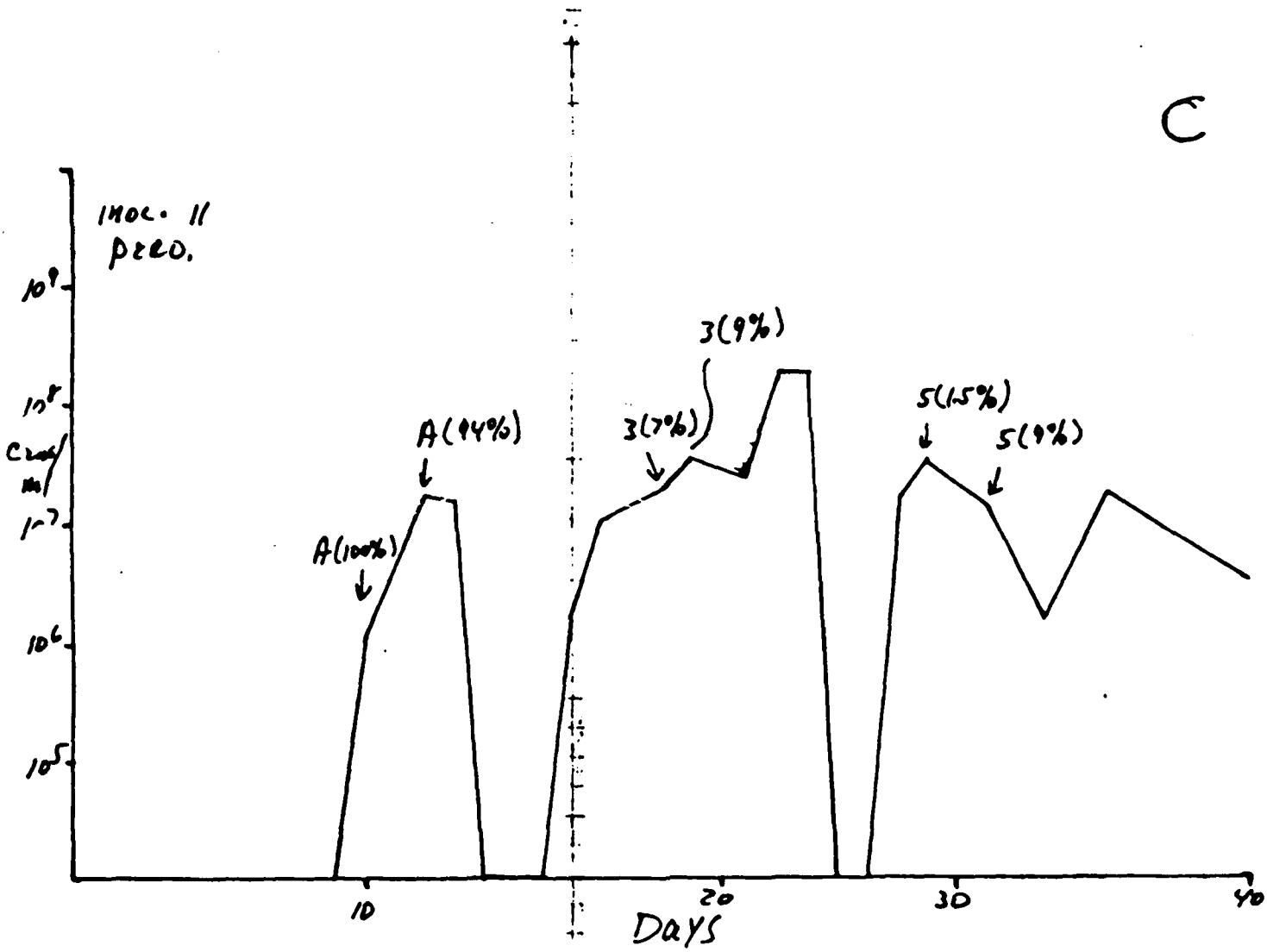


FIG. 1
Part 2 of 2



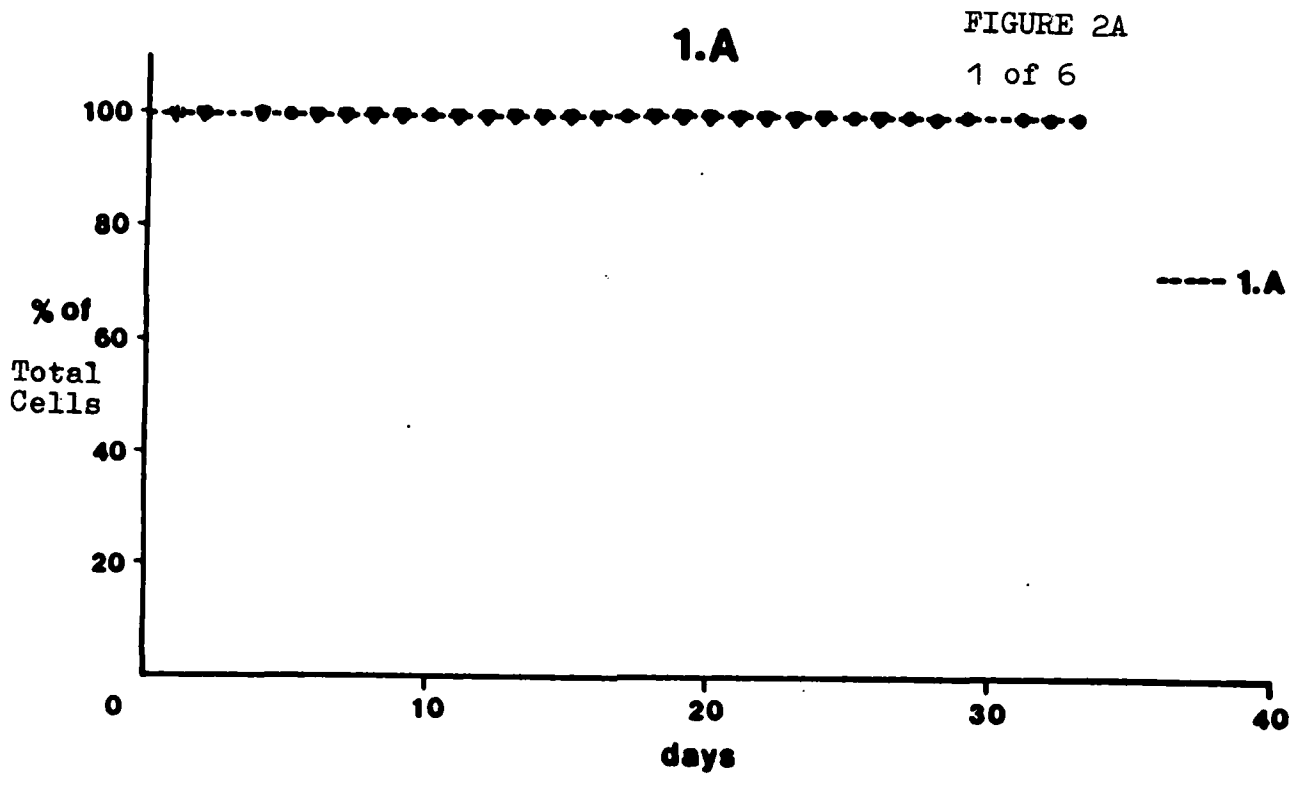


FIGURE 2A
1 of 6

FIG. 2B
2 of 6

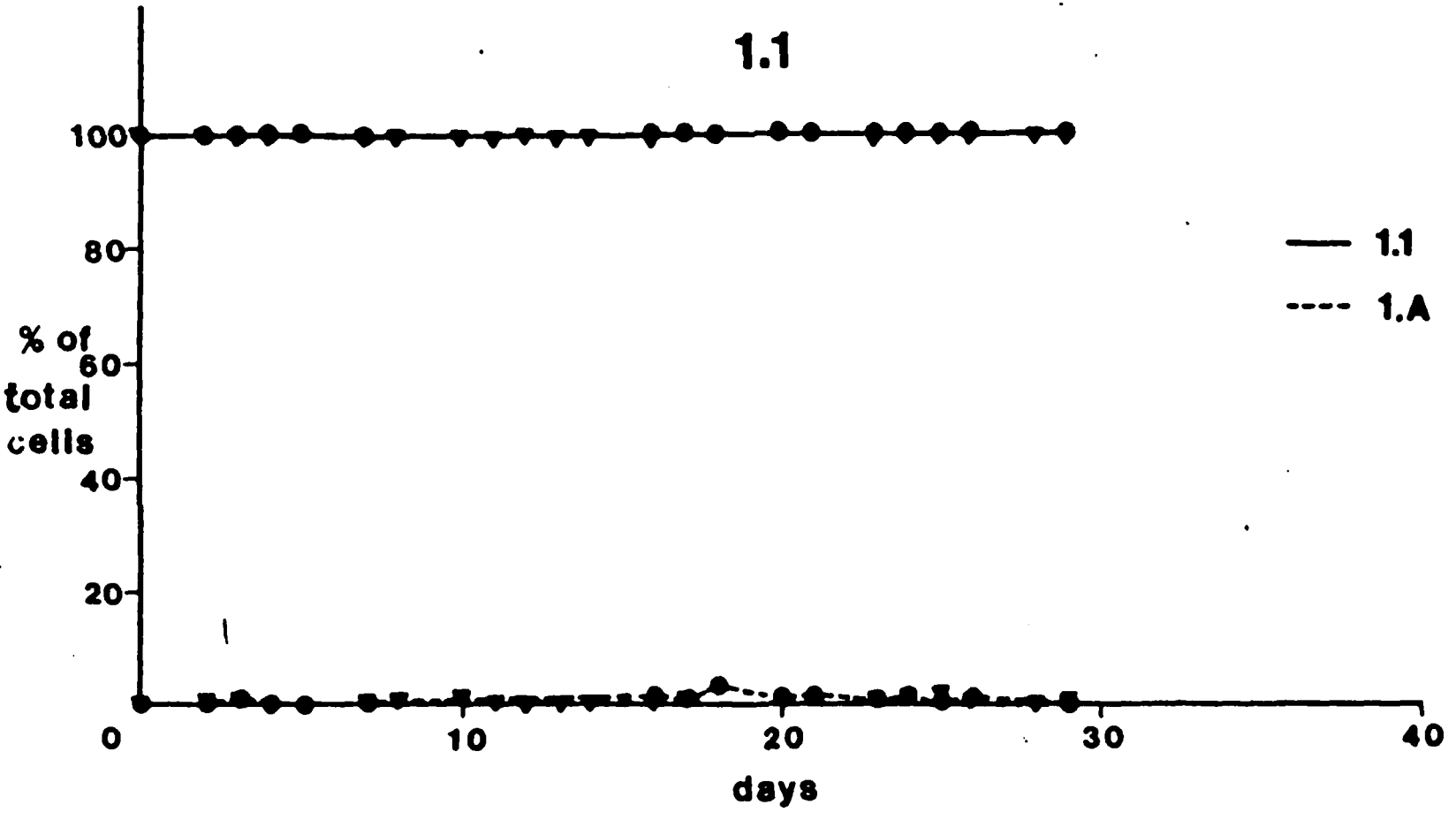


FIG. 2C
3 of 6

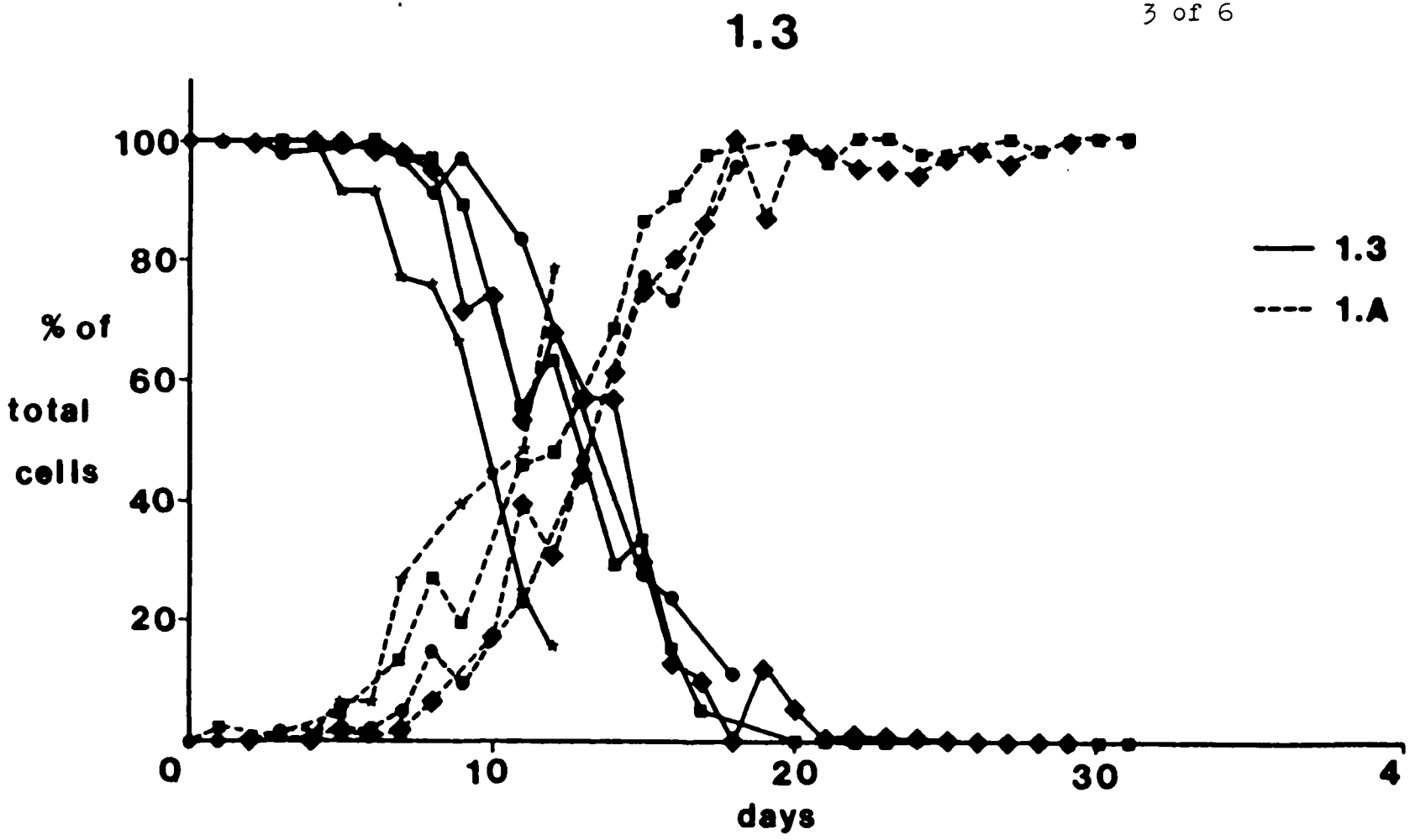


FIG. 2D
4 of 6

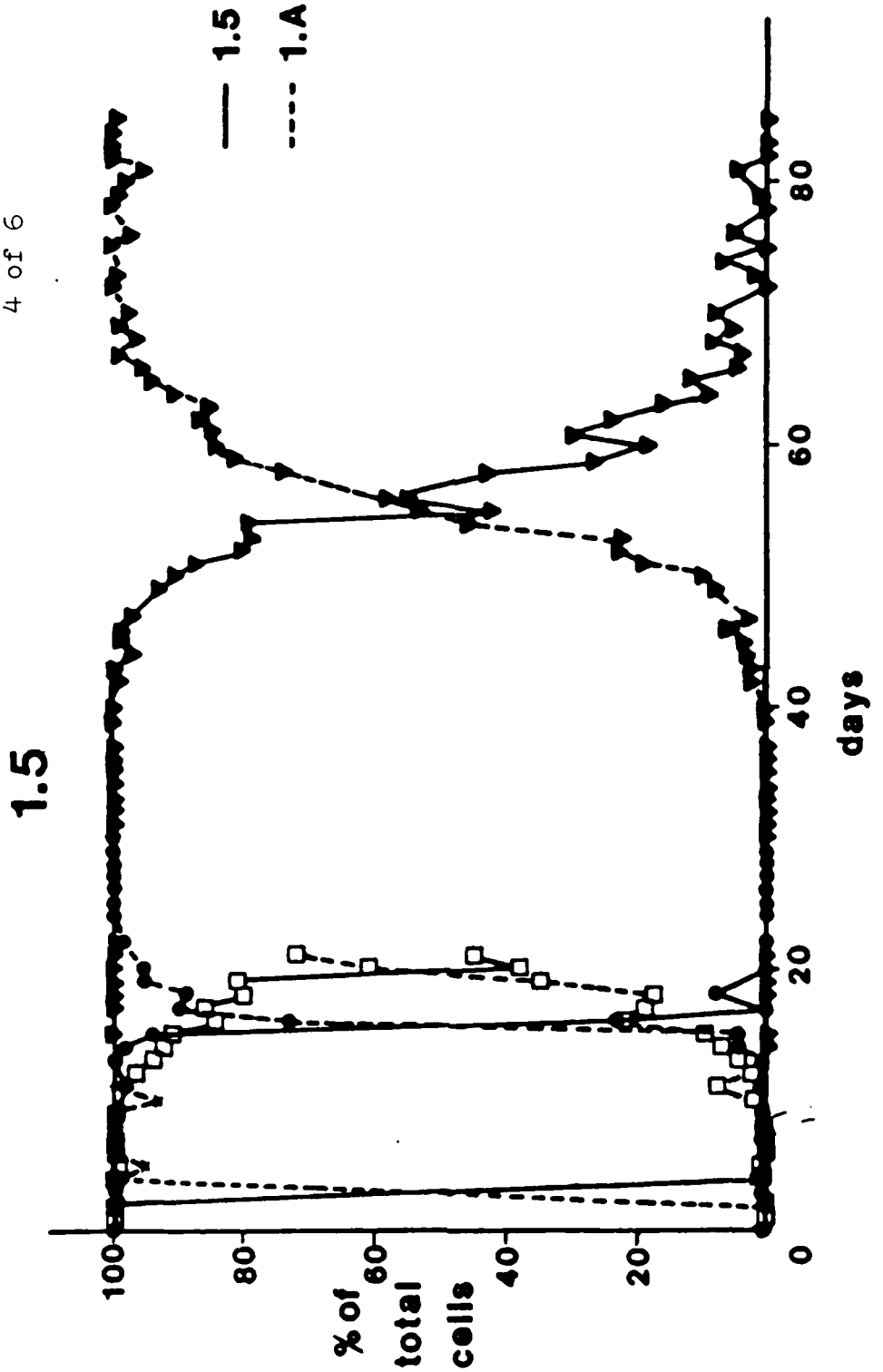


FIG. 2E
5 of 6

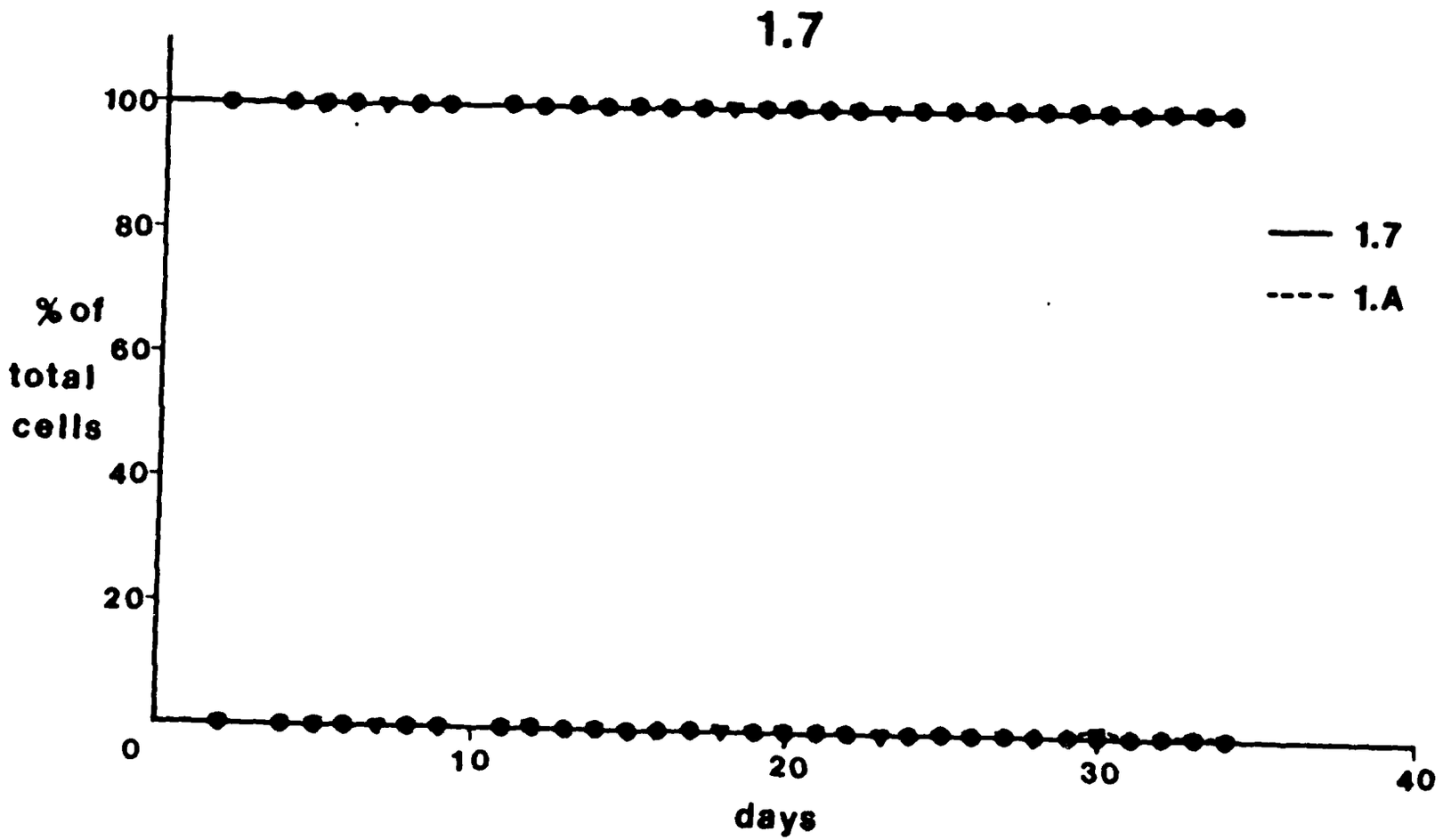


FIG. 2F
6 of 6

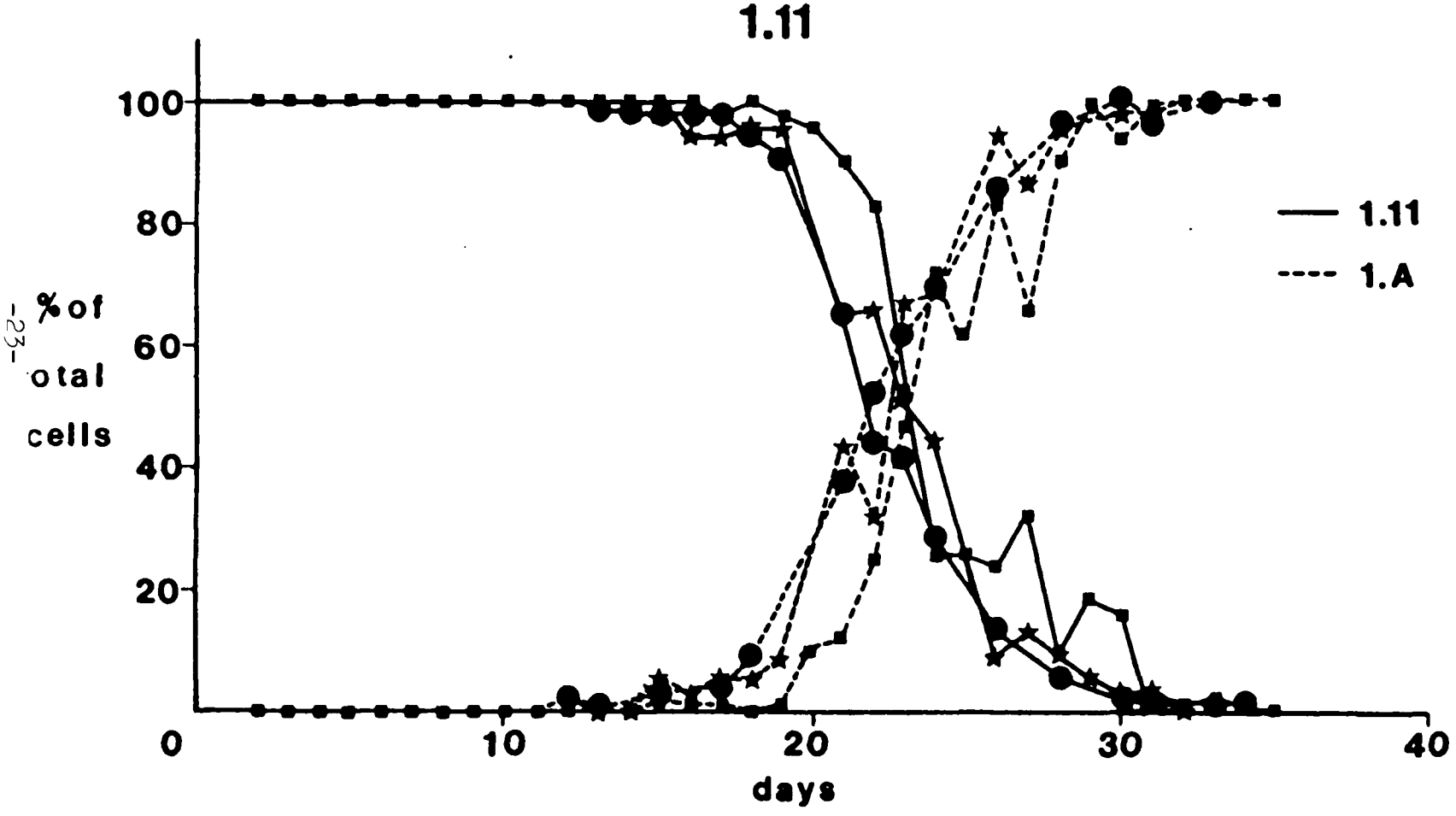
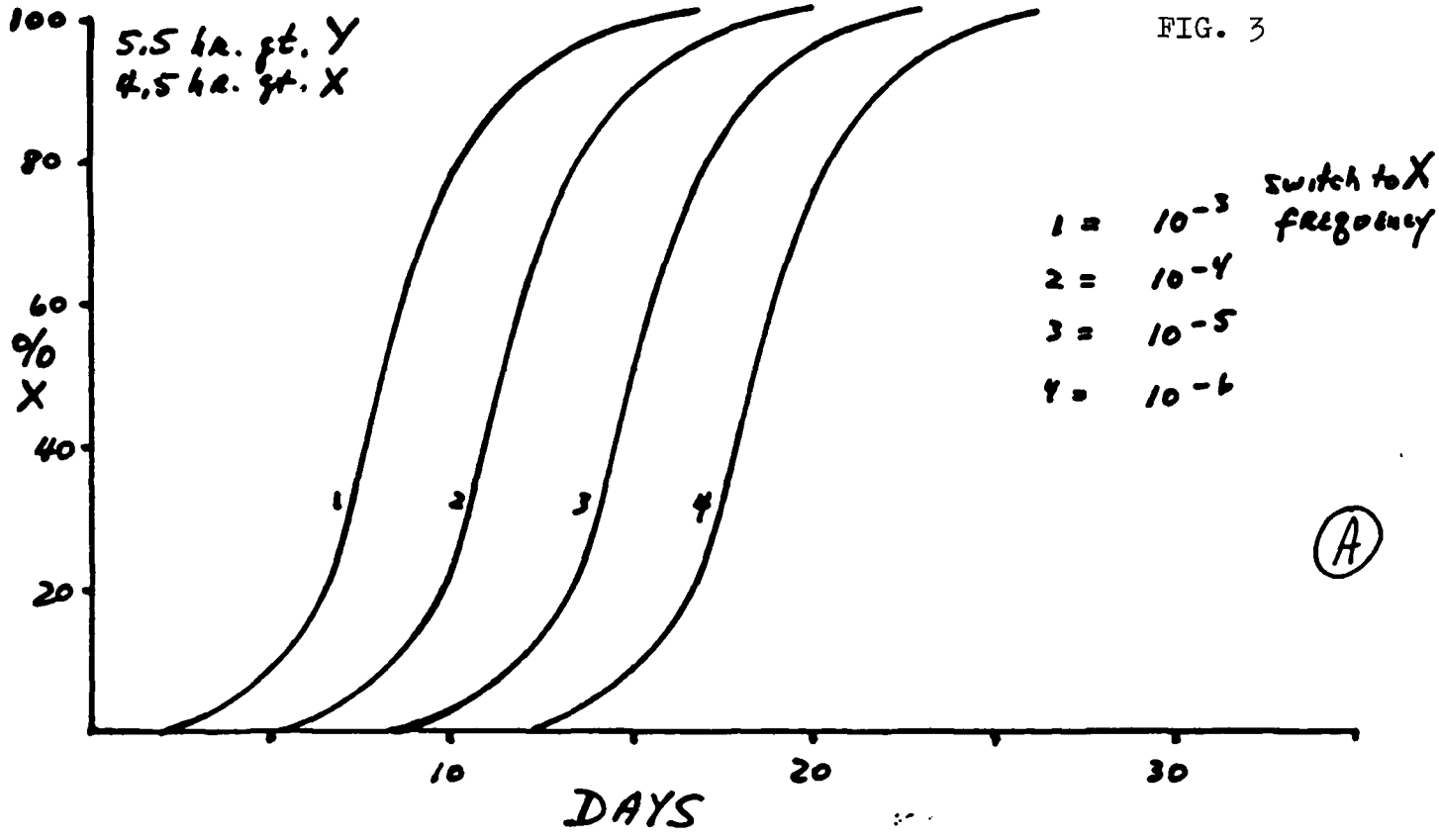


FIG. 3


5.5 hr. gt. Y
4.5 hr. gt. X
100
80
60
% X
40
20
1
2
3
4
10
20
30
DAYS
switch to X frequency
1 = 10^-3
2 = 10^-4
3 = 10^-5
4 = 10^-6
A

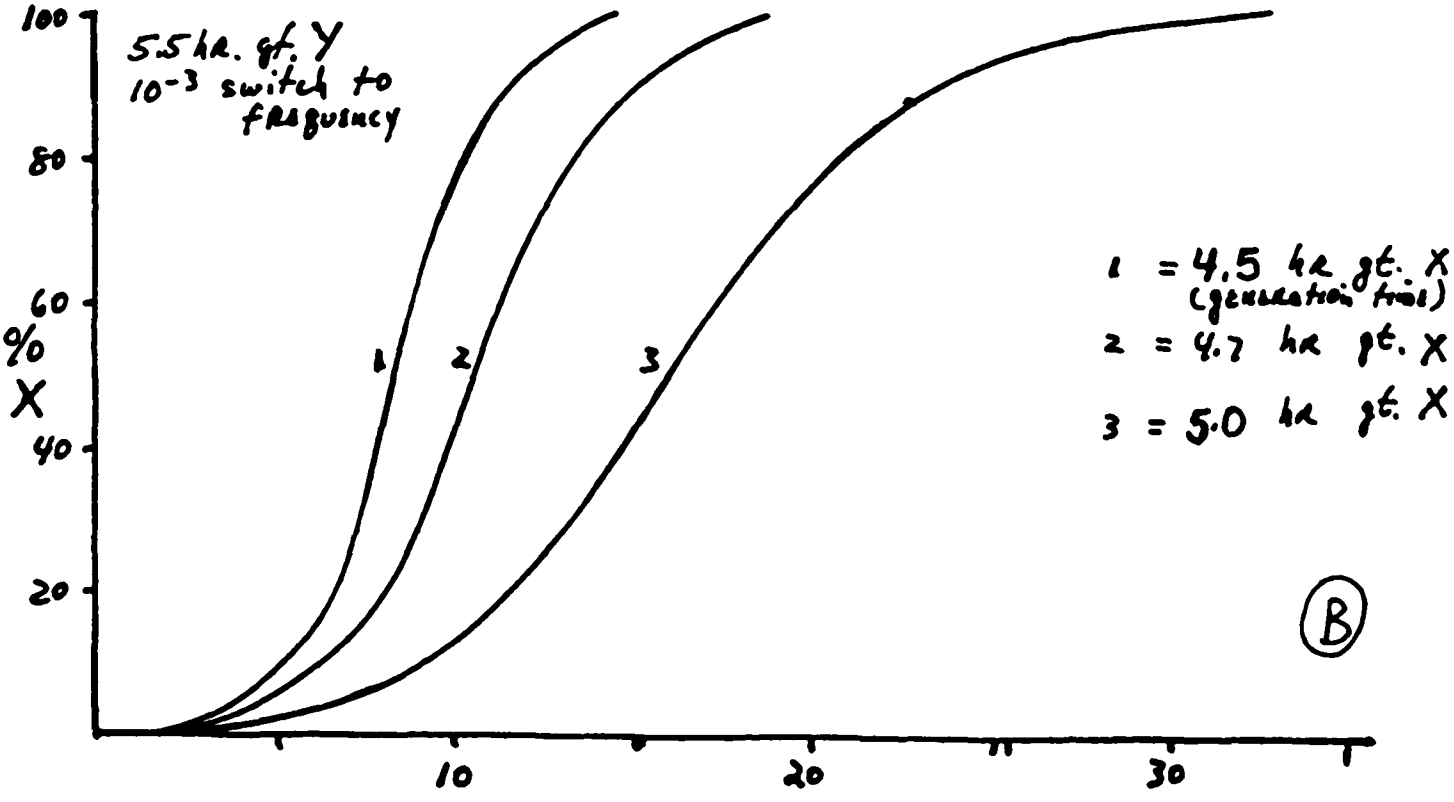
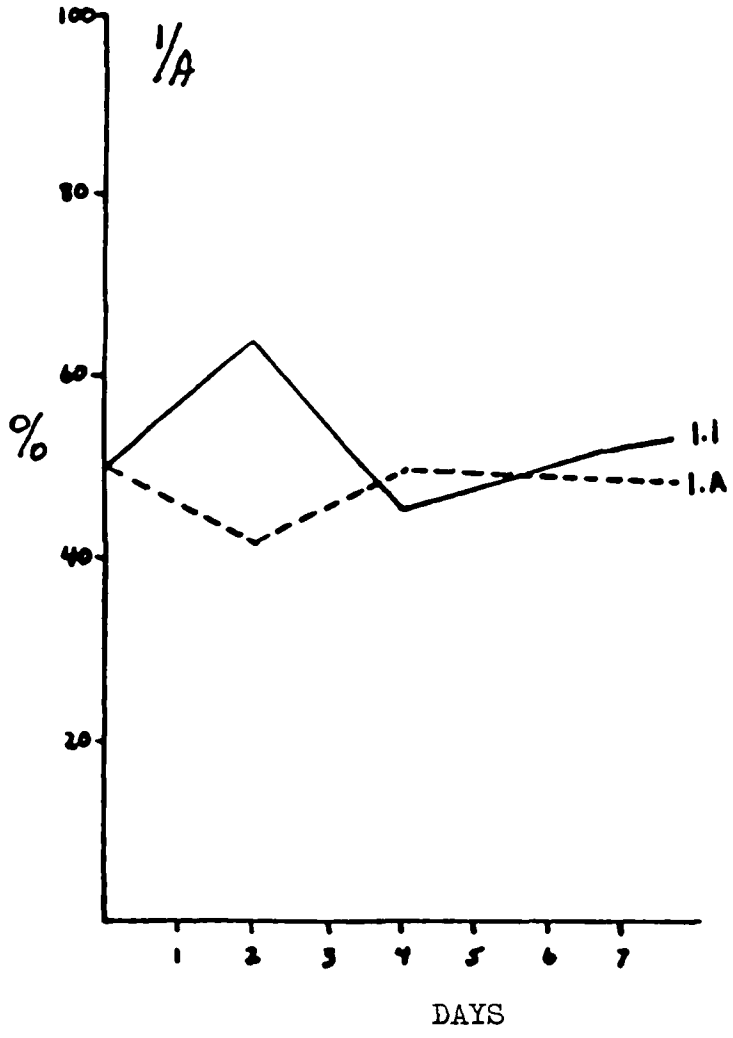

5.5 hr. gt. Y
10^-3 switch to frequency
100
80
60
% X
40
20
1
2
3
10
20
30
1 = 4.5 hr gt. X (generation time)
2 = 4.7 hr gt. X
3 = 5.0 hr gt. X
B

TABLE 1

Growth rates of different T.brucei VATs

VAT	generation time (hours)	"competition" with 1.A	change to 1.A (days)
1.A	4.4-5.2	-	-
1.1	4.9±0.5	equal	50
1.3	5.5±0.5	slower	20
1.5	5.0±0.2	slower	5-20
1.7	4.7±0.3	faster	>35
1.11	5.4±0.4	slower	30

FIG. 4
Part 1 of 3



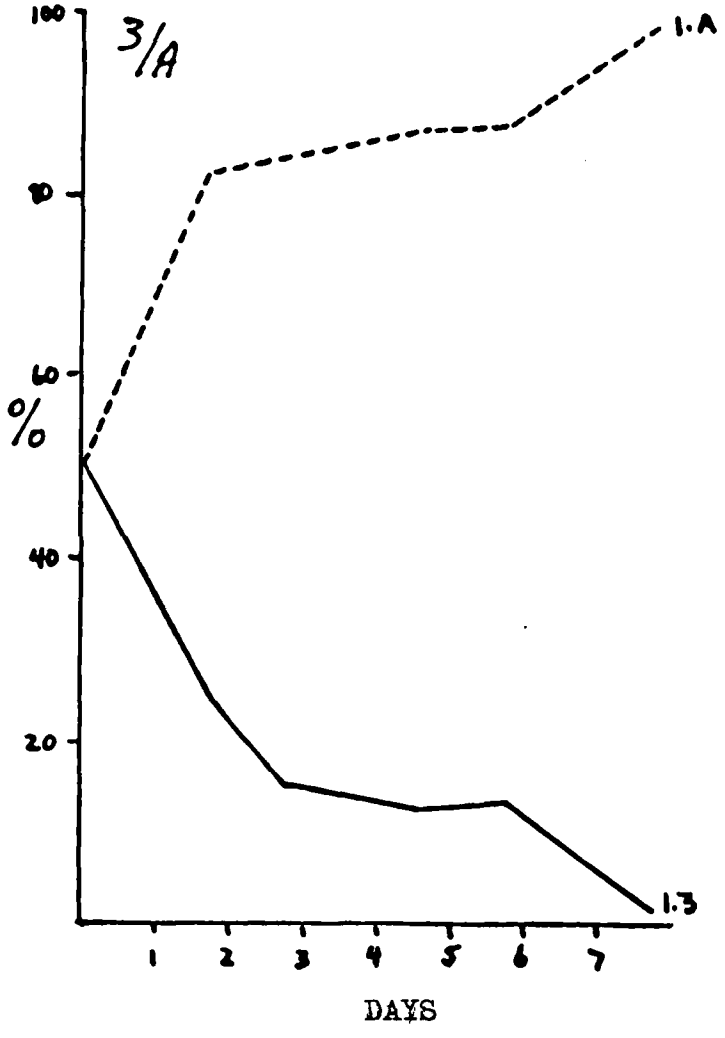


FIG. 4
Part 2 of 3

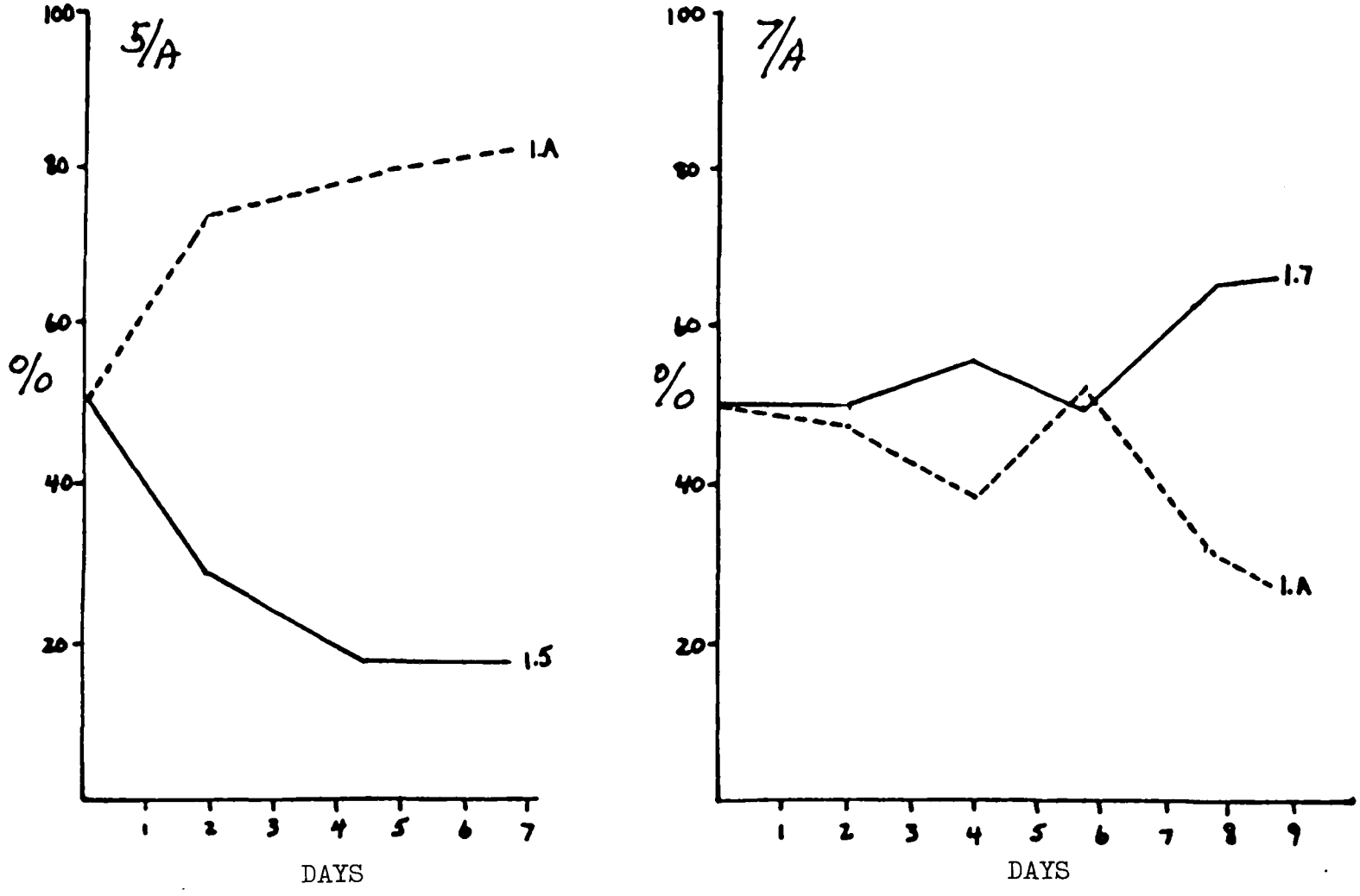
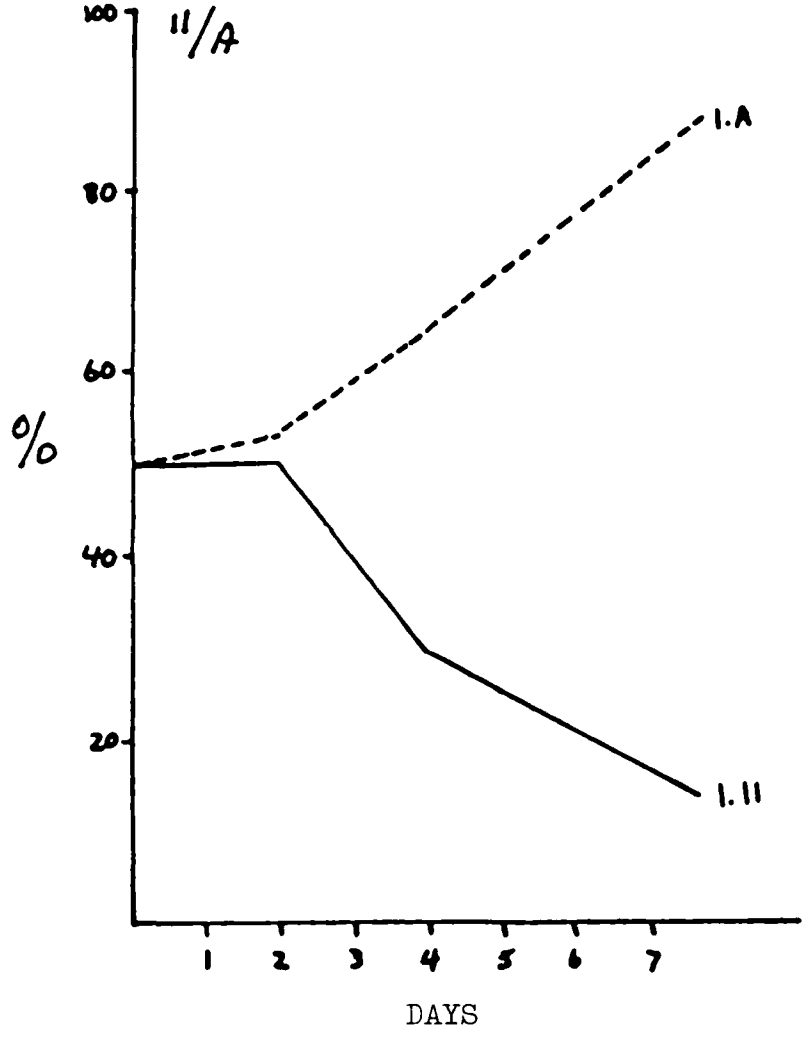


FIG. 4
Part 3 of 3



SPECIES	IHRI STOCK #	REACTION WITH VSG SPECIFIC McABS A	1	3	5	7	11	STOCK TYPE	CLONE	ORIGIN
		100	-	-	-	-	-			
T. brucei	1	-	2.9	2.1	2.8	2.4	-	lab	+	Tanzania
"	2	-	+	+	-	-	-	field	-	"
		-	-	-	-	1.0	-			
"	3	+	+	+	+	+	+	field	-	"
		-	-	3.8	3.4	1.1	+			
"	4	-	-	-	-	+	-	fly	+	"
		+	-	-	-	-	-			
"	5	+	-	-	-	-	-	field	-	Kenya
		+	+	+	1.2	+	-			
"	6	3.1	+	+	-	+	+	fly	-	Uganda
		-	+	-	+	-	+			
"	7	-	-	-	-	-	-	lab	-	"
		+	-	+	-	-	-			
"	9	+	-	-	-	-	-	lab	+	Kenya
		-	-	-	-	-	-			
"	10	-	-	-	-	+	-	lab	-	"
		-	-	-	-	-	-			
"	11	-	-	-	-	+	-	lab	-	"
		1.0	2.3	+	-	2.8	-			
T. gambiense	12	-	-	-	-	-	-	field	+	Zaire
		7.8	-	+	7.0	-	-			
"	13	52.0	-	-	-	+	-	field	+	"
		not done								
"	14	+	-	-	-	-	-	lab	-	Nigeria
		not done								
"	16	+	-	-	-	-	-	lab	-	"
		not done								
"	17	-	-	-	-	-	-	lab	-	"
		not done								
"	18	-	-	-	-	-	-	field	-	Kenya
		not done								
T. rhodesiense	20	+	1.0	-	-	-	-	field	-	"
		-	-	5.6	4.0	8.0	-			
"	21	-	-	-	-	+	-	lab	+	"
		10.0	12.8	-	16.3	32.3	-			
"	22	-	-	+	-	+	-	lab	+	"
		not done								
T. evansi	23	-	-	-	-	-	-	lab	+	?
		-	-	-	-	-	-			
"	24	-	-	-	-	-	-	lab	+	?
		-	-	-	-	-	-			
T. equiuum	25	-	-	+	+	-	+	lab	+	?
		1.5	+	1.3	+	1.0	-			
f/%		15/68	9/41	11/50	8/36	13/59	3/14			

TABLE 2: Expression of IsTaR 1 like VSGs in other stocks. Values are percent of population (+= less than 1%). f refers to frequency of VSG expression in stock or its relapse. Top line refers to first parasitemia while bottom is first relapse in each case.

TABLE 3. Reactivity of VSG-specific monoclonal antibodies

VSG*	no.	class	VAT 1.A	1.D	1.1	1.3	1.5	1.7	1.11	live cell IFA	Western+	pptn. *in vitro*#
A	4	G	**+++**/+	−/−	−/−	++/−	−/−	−/−	−/−	0/4	4/4	3/3
1.1	8	G	−/−	**+++**/++	**+++**/++	−/−	−/−	−/−	−/−	0/7	7/7	2/2
1.3	1	G	**+++**/+	−/−	−/−	++/−	−/−	−/−	−/−	0/1	1/1	
1.3	14	G	−/−	−/−	−/−	**+++**/+/−	−/−	−/−	−/−	10/14	1/14	2/6
1.5	16	G	−/−	−/−	−/−	−/−	**+++**/+	−/−	−/−	1/16	16/16	7/7
1.7	8	G	−/−	−/−	−/−	−/−	−/−	**+++**/+/−	−/−	1/8	1/8	
1.11	4	G	−/−	−/−	−/−	−/−	−/−	−/−	**+++**/+	4/4	0/4	1/2
1.11	1	G	−/−	+/−	++/−	−/−	−/−	−/−	**+++**/++	1/1	0/1	

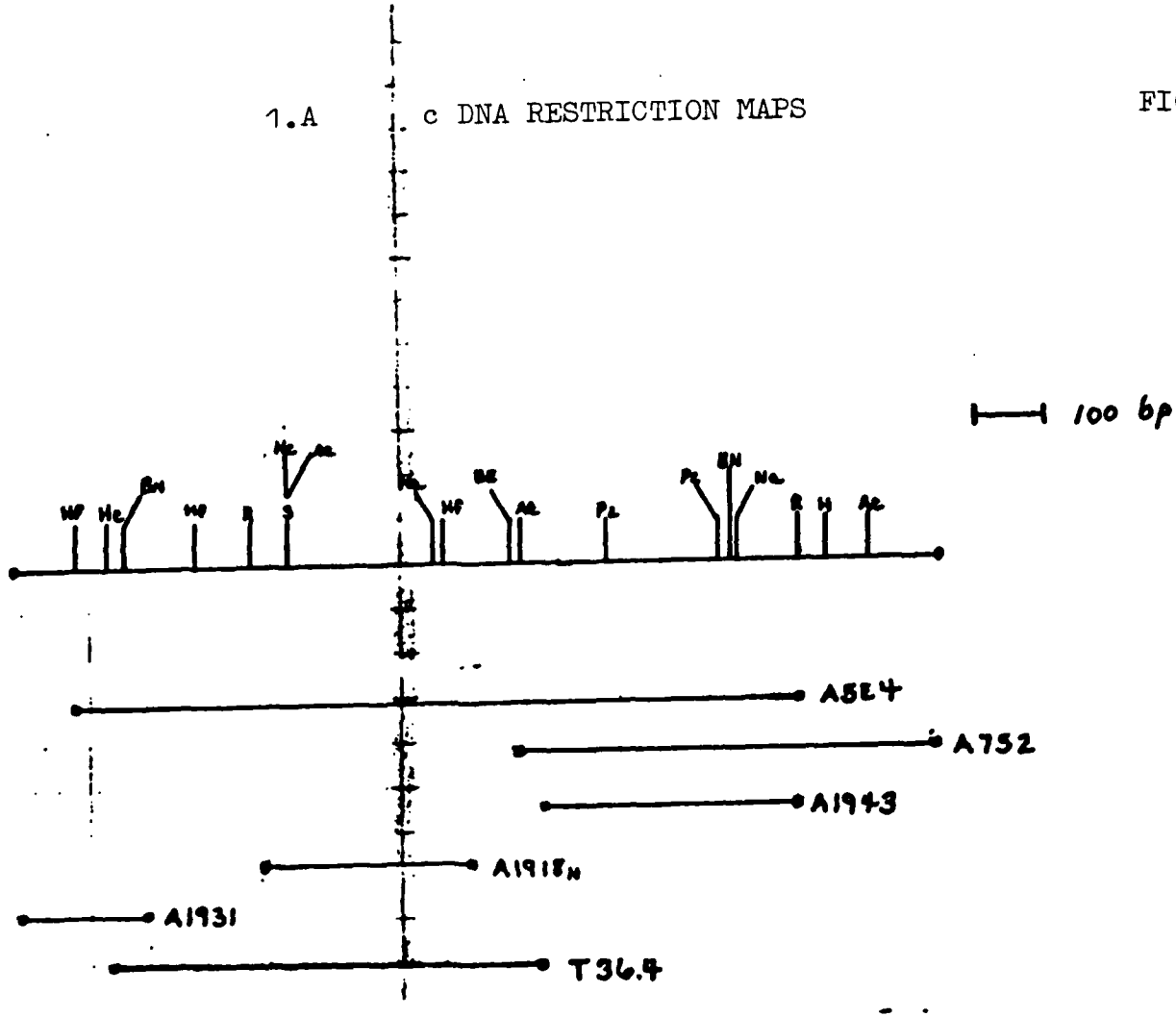
+++/+ reaction in IFA with acetone fixed cells
reaction in EIA using purified VSG
reaction in EIA using whole cell lysates

+++ titer > 10^5
++ titer = 10^3 - 10^5
+ titer = 1 - 100
+/− titer = 1
− no reaction at any dilution

* VSG used to immunize the mice before fusion
\+ reactivity in immunoblot analysis with SDS/2-mercaptoethanol denatured VSG
\# immunoprecipitation of *in vitro* translated VSG

1.A c DNA RESTRICTION MAPS

FIG. 5A

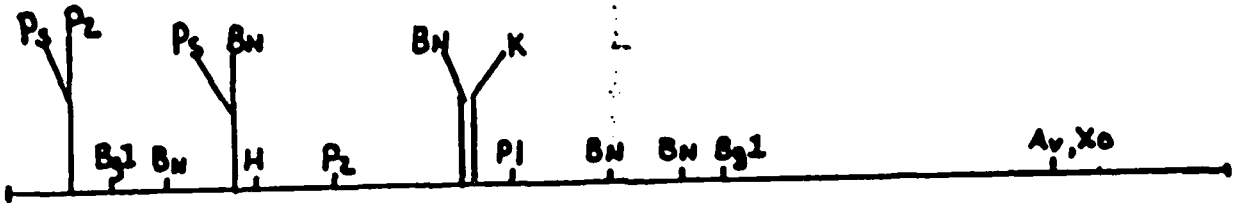


Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	P1=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bg1=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

1.1 cDNAs FIG. 5B

100 bp

● = 35 mer sequence



J97D

J16F

J44D

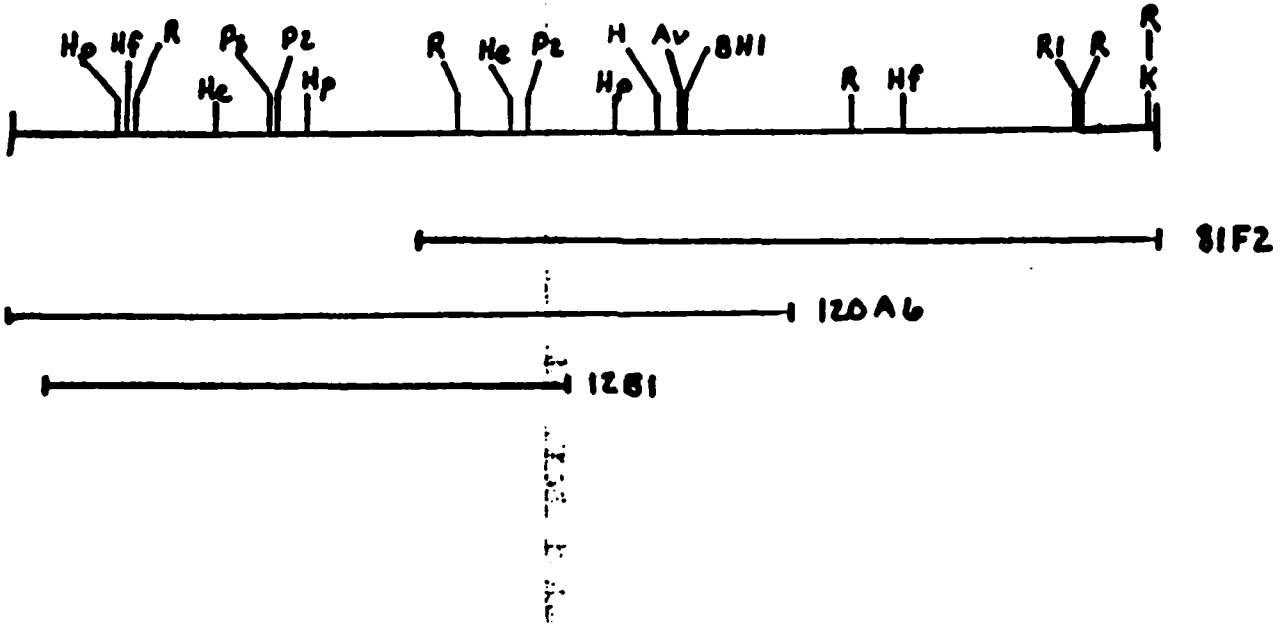
D4F5

D

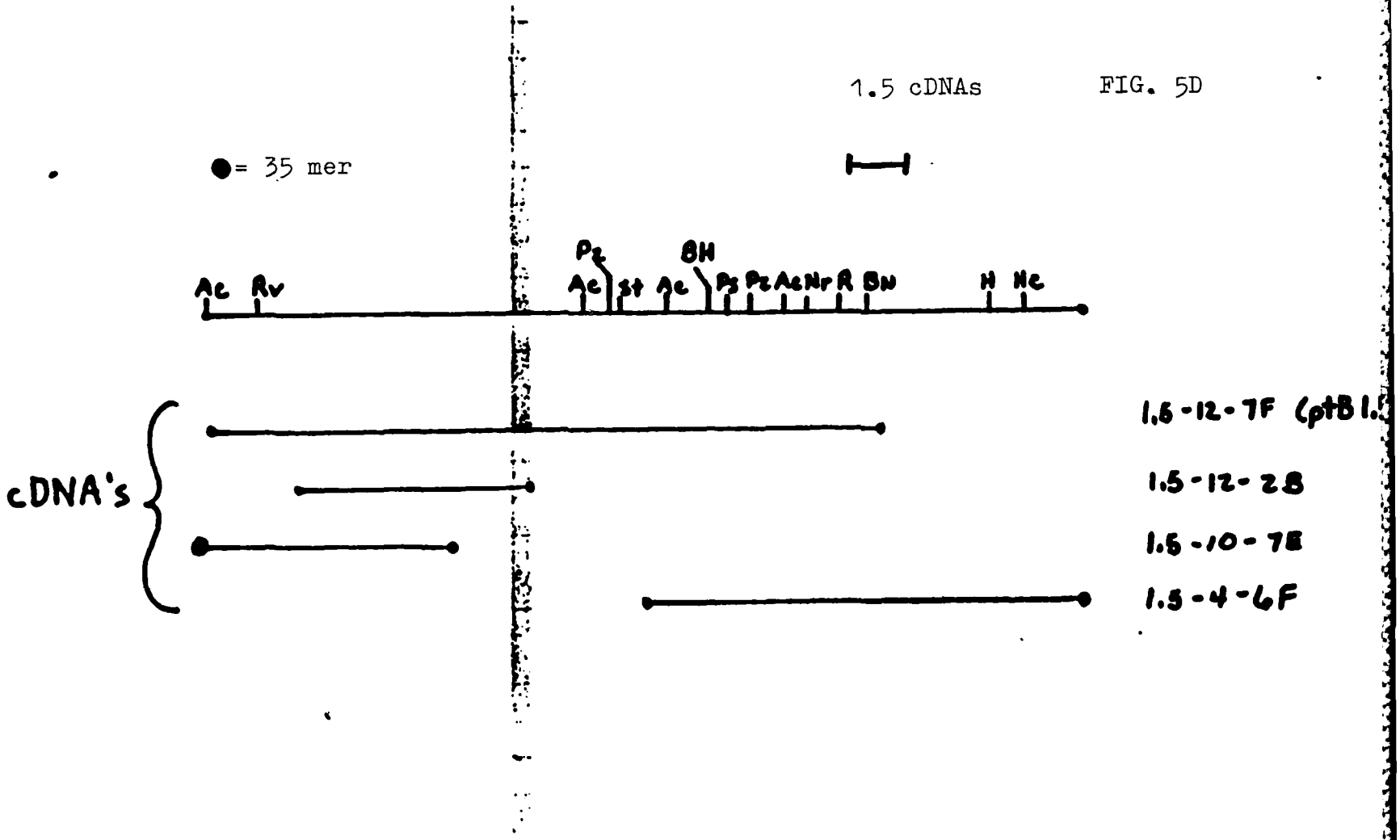
~ poly A tail

Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	P1=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bg1=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

FIG. 5C



Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I



Ac=Acc I
Al=Alu I
Av=Ava I
BHI=BamHI
Bg1=Bgl I
Bg2=Bgl II
BE=BstE II
BN=BstN I
CL=Cla I
RI=EcoR
RV=ECORV
He=Hae III
Hc=Hinc II
H=Hind III
Hh=Hha I
Hf=Hinf I
Hp=Hpa II
K=Kpn I
Na=Nae I
Nr=Nar I
Pl=Pvu I
P2=Pvu II
Ps=Pst I
R=Rsa I
S=Sal I
Sm=Sma I
Sp=Sph I
St=Sst I
T=Taq I
Xo=Xho I

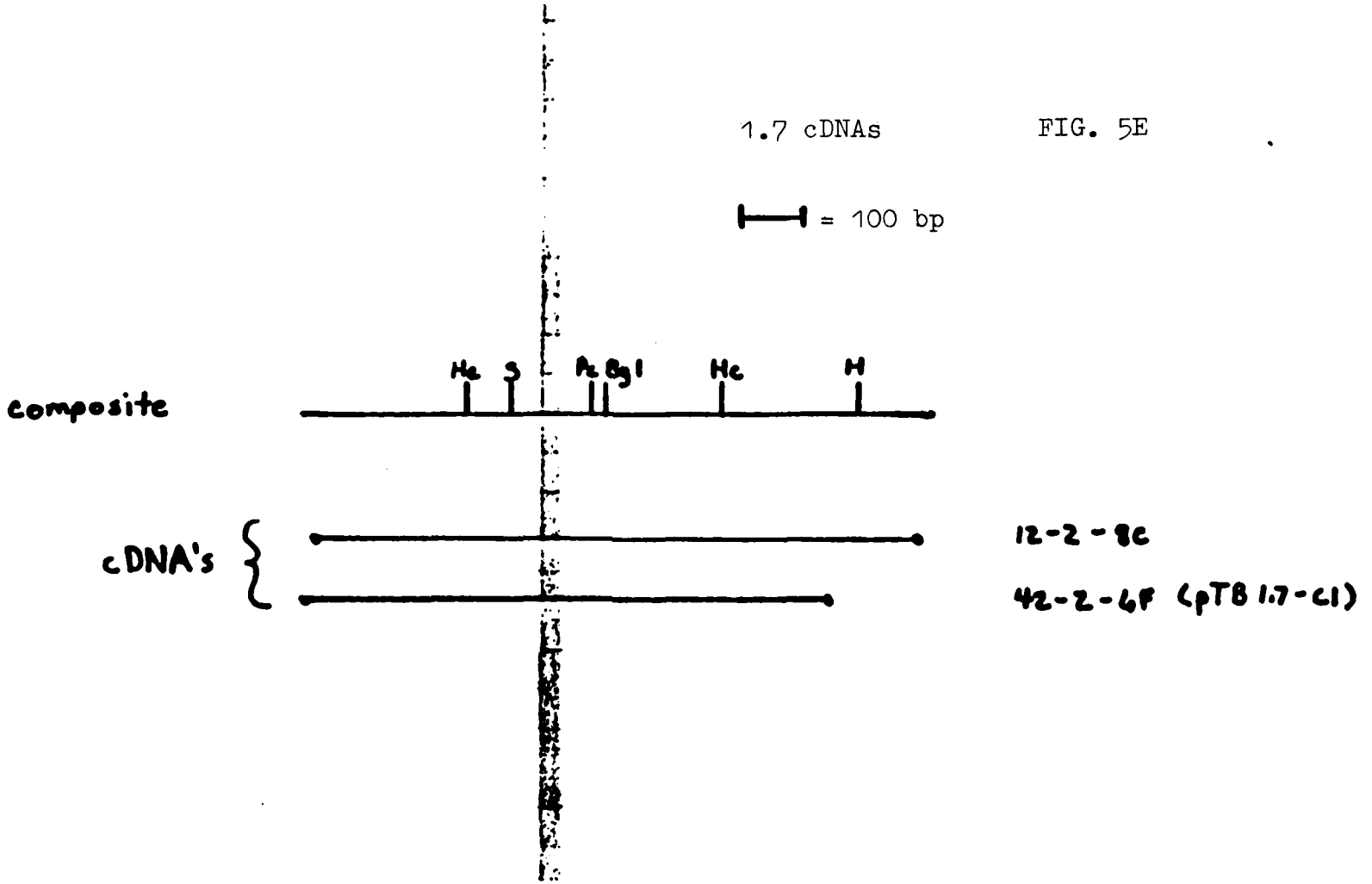
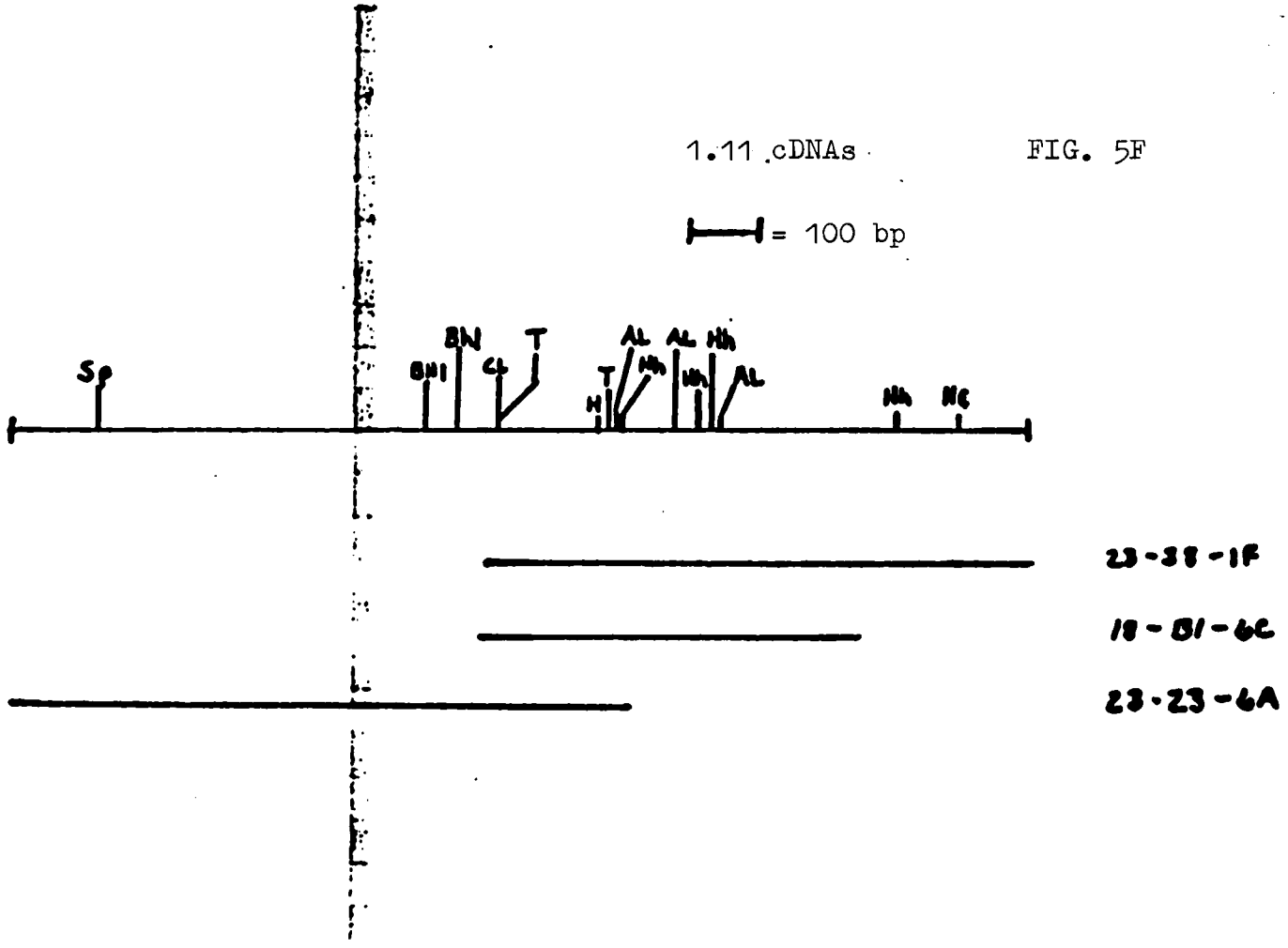


FIG. 5E

Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

1.11 cDNAs FIG. 5F



Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

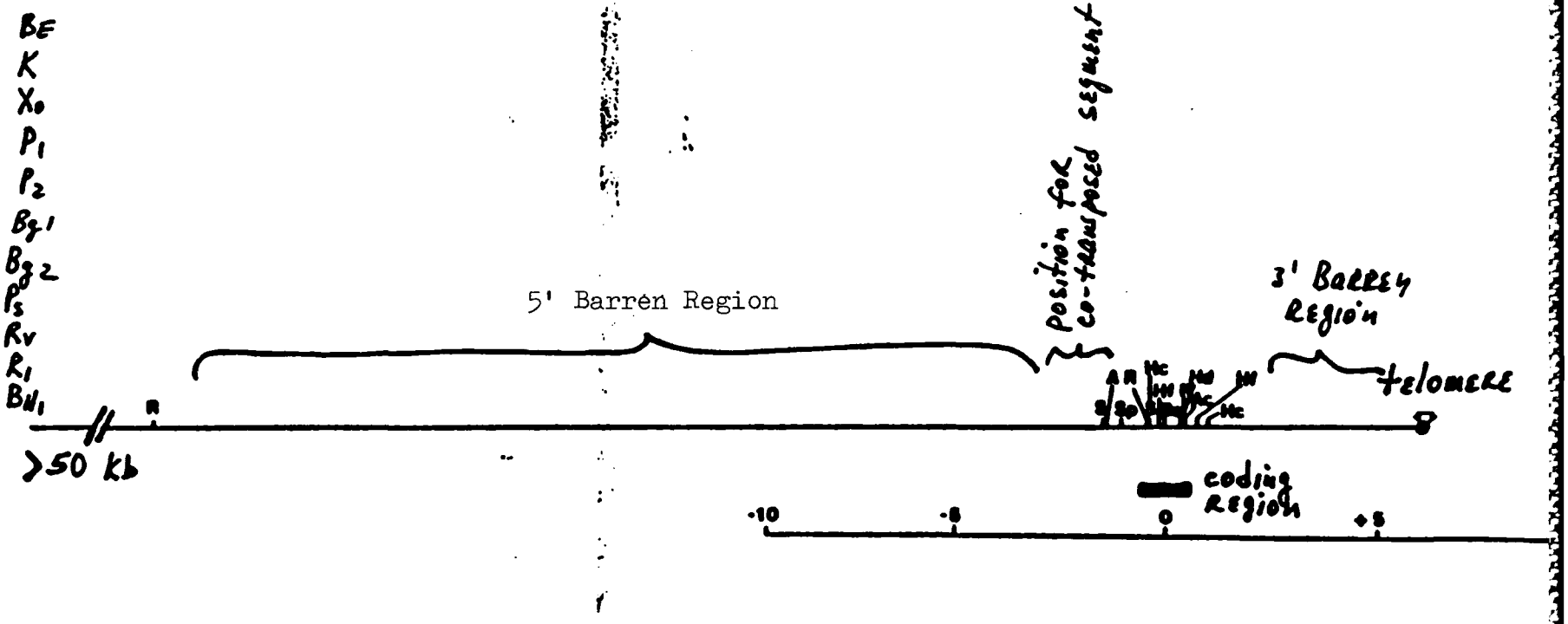
1.A ELC

1 kb

FIG. 6A

1 of 7

O = Bst EII



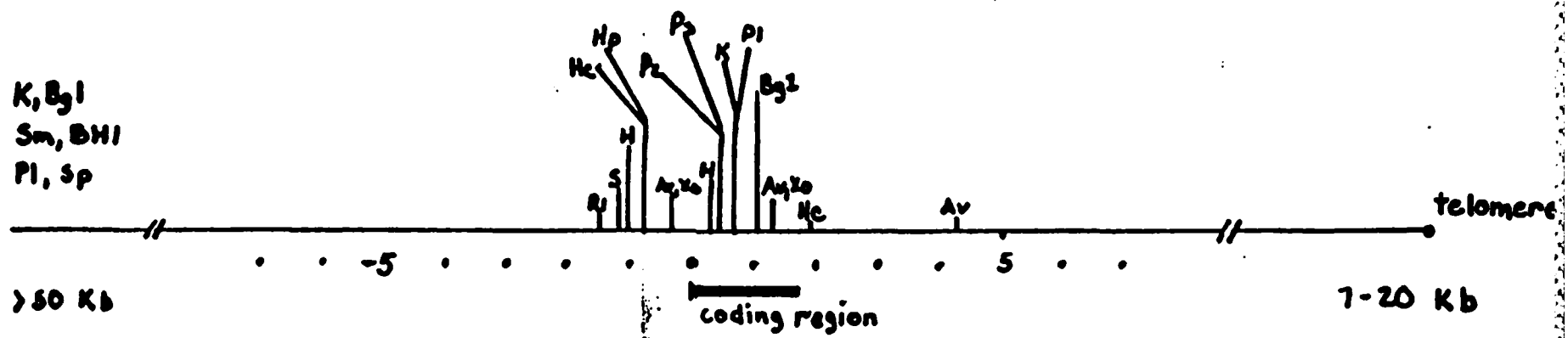
Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	P1=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bg1=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

FIG. 6B 2 of 7

1.1 Gene ELC

O = 3' to 35 mer sequence in c DNA

1 kb

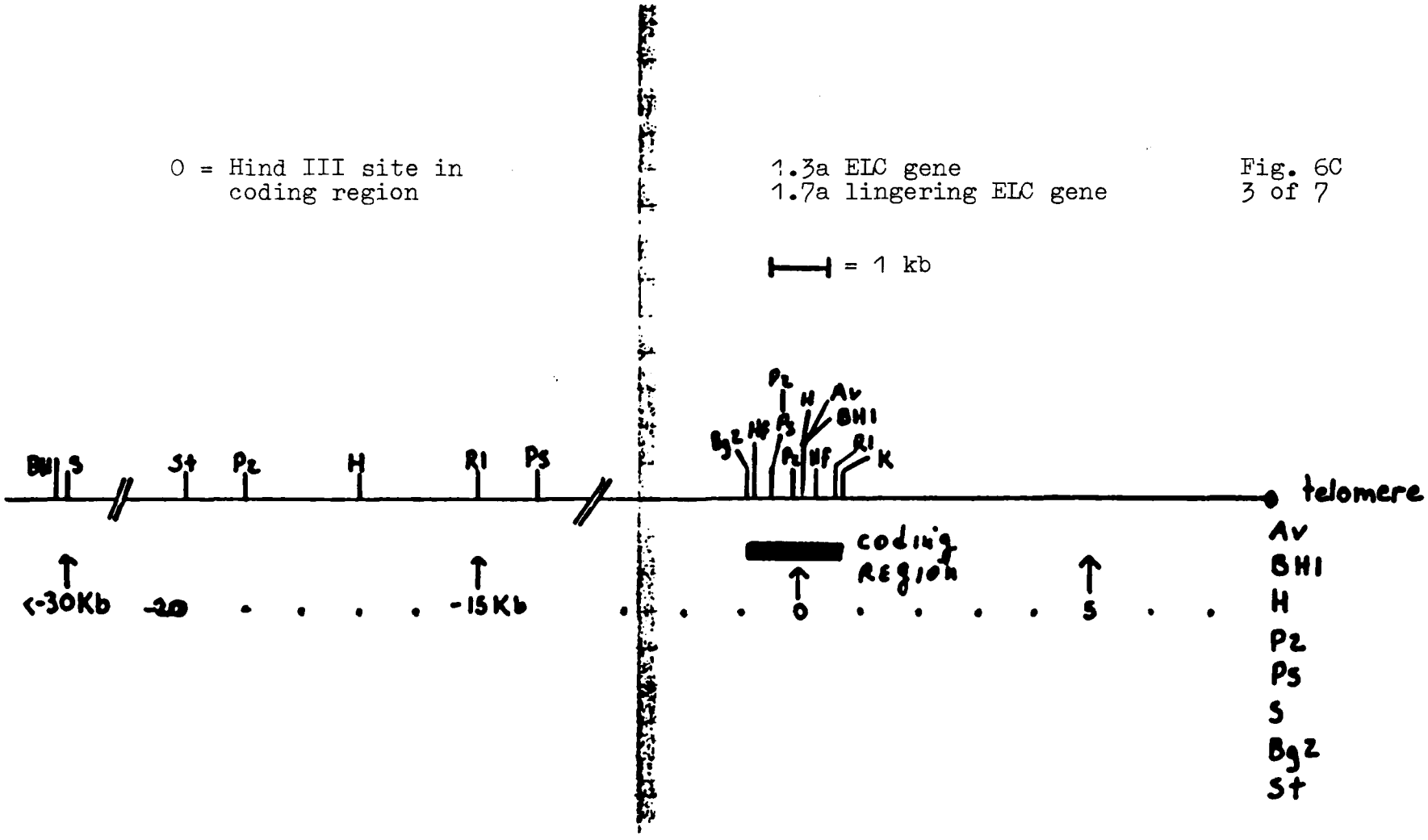


Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

O = Hind III site in coding region

1.3a ELC gene
1.7a lingering ELC gene

Fig. 6C
3 of 7



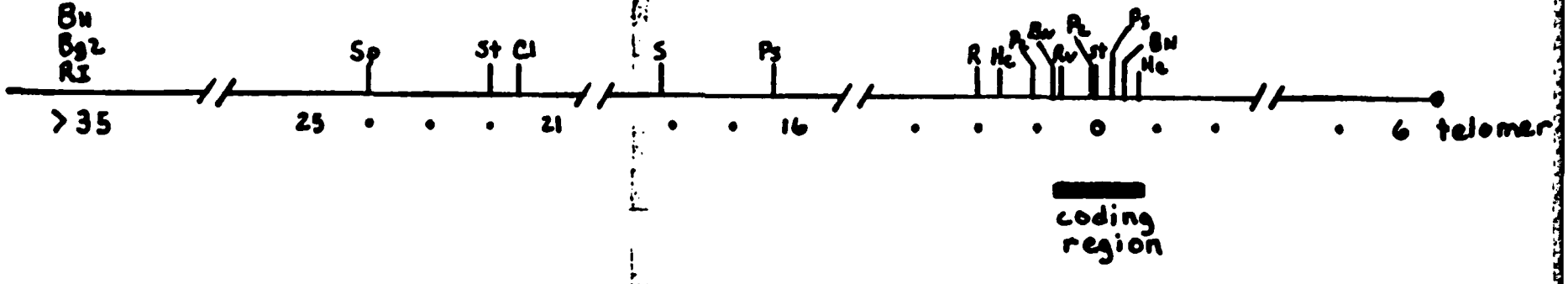
Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

FIG. 6D
4 of 7

O = SstI site in coding region

1.5 telomeric gene
Expression linked copy (ELC)

= 1 kb

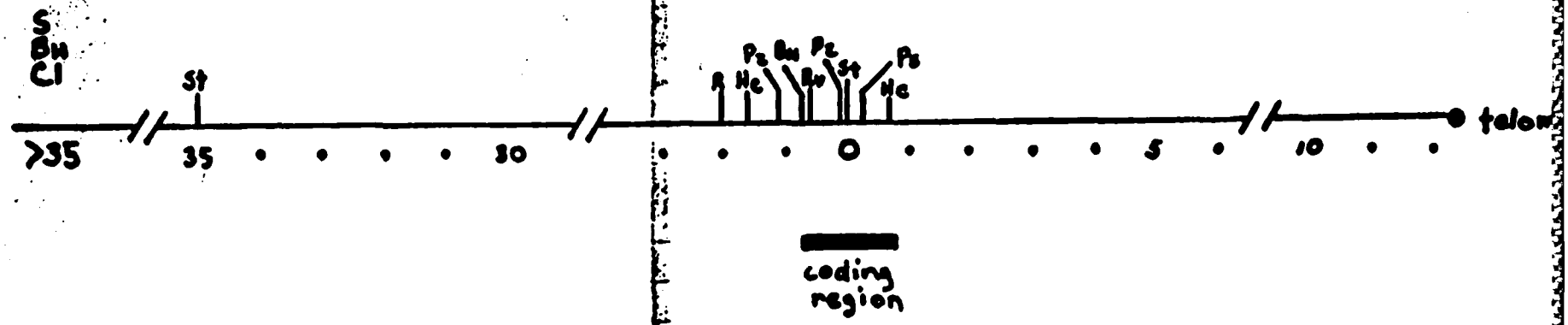


Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	P1=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bg1=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

1.5 telomeric gene
(not expressed)

= 1 kb

O = SstI site in coding region



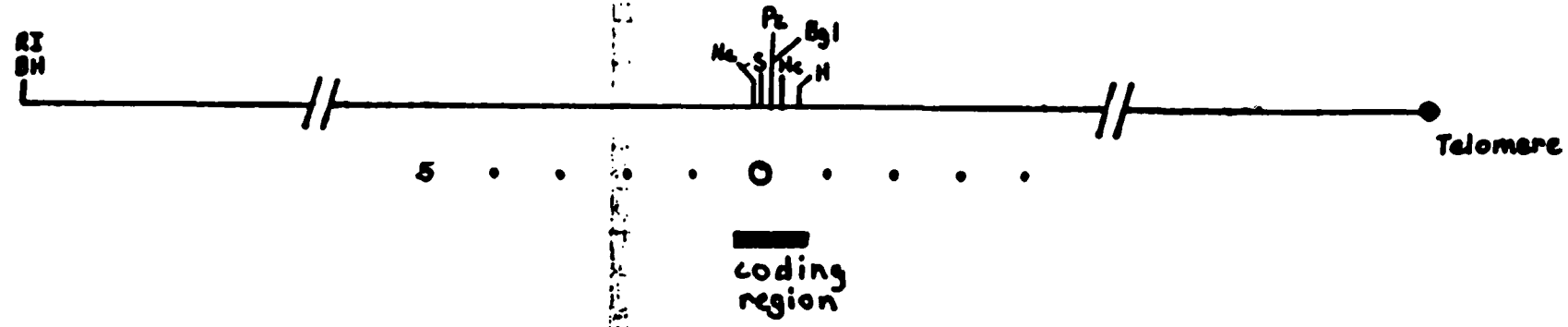
Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

FIG. 6F
6 of 7

1.7 telomeric gene
Expression linked copy (ELC)

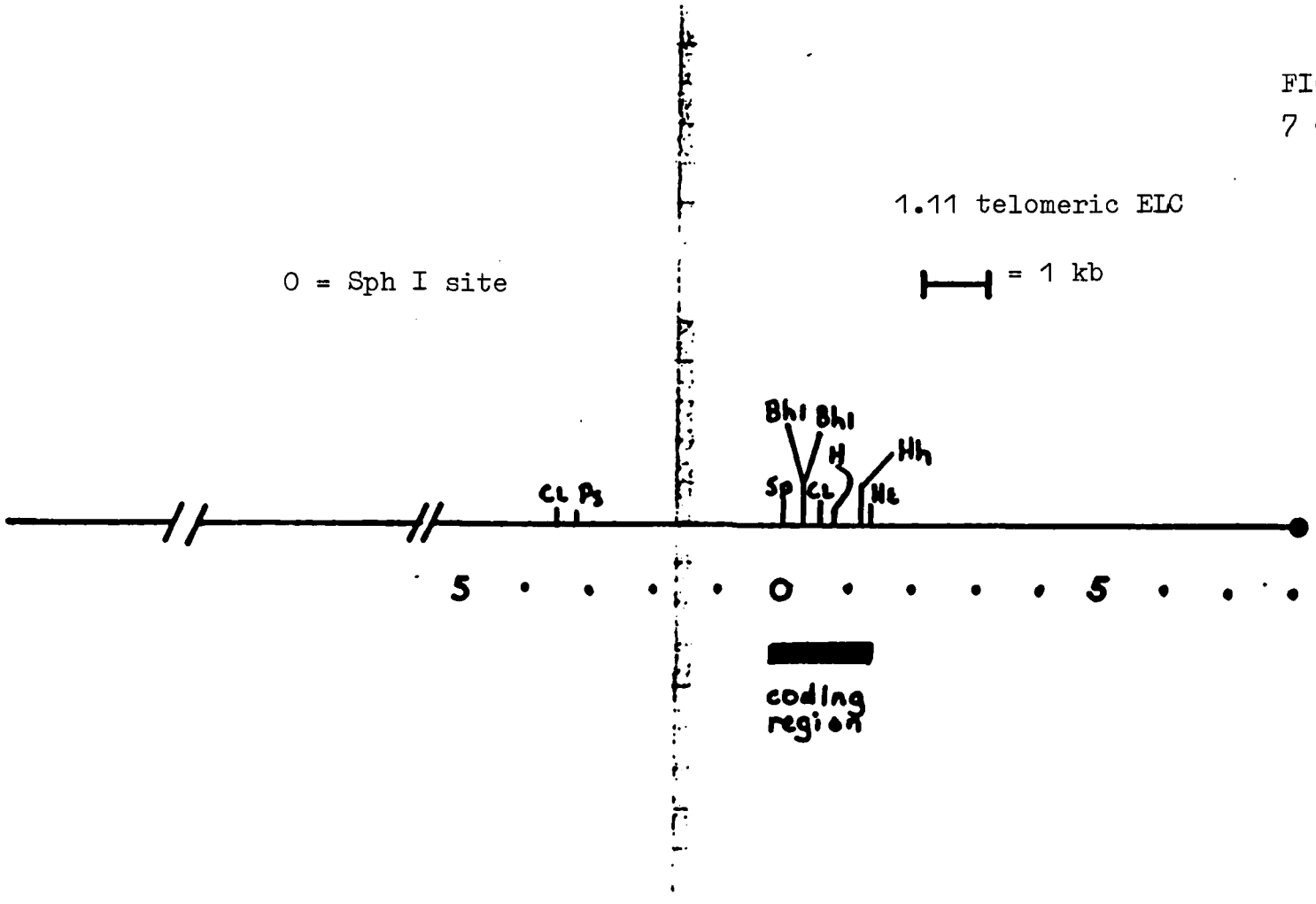
O = Sal I site in coding region

— 1 kb



Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

FIG. 6G
7 of 7

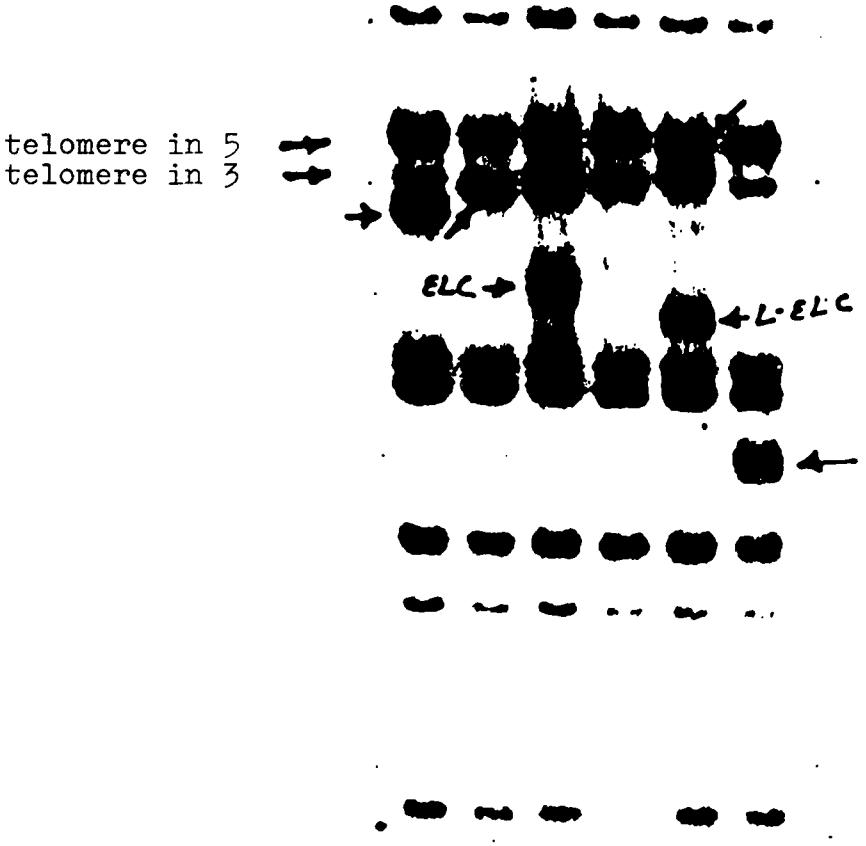


-43-

Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	P1=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

FIG. 7

A 1 3 5 7 11 Probe P/B 81F2 3 cDNA
Hind III digest



unlabeled = telomere

FIG. 8

VSG gene 1.11 sequence: 35-MER

CGCTATTATTAGAACAGTTTCTGTACTATATTGACTCGGCTGCAAACAAC
TAAGACACAGAAAACAAGCTGCCTTGAATAAAATAGAAAGCGCTAAAGAG... INIT CODON

TGAGGCTATCTGCA...CACGCCAGATATAATCGATAATAATATAACATC
ACTAGCAGAAGCAGCCTCAACACTTGAAAATTTGCAAGACTTAACAAGCA
TCGCCAGTTACAAACAGGATGCCTTGTTTCAAAGACTGGTAGCAATAACA

AAACTCGGAATGCCAACAGATTCAAAGCTTACTGGCGACCTCGAACGCCA
GGTAAGCTCAGCAATCAGCAGCGCATACGGCAAAACTGACAGCGAATTTC
AAGACAAAATTTGGAGGCAACTTGACGAACTAGATTCCAACTATTACAGC

TCCTTAGGGGTTAAATCAGACAAAATTAAAGGTTTGACCAGCGCTGAGAA
AATGGCTGGCGCCATAGCTTGGGGTTTAATTAACAAAATTAGTGCCAAAC
AATCATTGTCCACCGACAACTTAGCAAACAAAAAGGACCTCAGCACAGTG

TGCAAAAACCATGTAACAAGAAATGATTGCACGGGAGAAACGGGTTGTGA

ATTTGACGAAACCAAAACTCCAAAATGCTTTCCAAAACCGAGTGAGACCA
AAGAAGAGACCAAAGAAAAGAAAG...CAAGAAAGAAGATGCAAACTGAC

TCACTGCACAGGAAAGAACAAAAGAATGCGCAAATGGTTGCAAATGGAGA
ATAATGCTGCAAAGATTCCTCTATCTCCCTAAACAAGAAATTCGCCCTCA
GCATGGTTTCTGCTGCATTTGTGGCCTTGCCTTTTTGAAACAACATTTTC TGA= STOP CODON

CCCCTCTTTTTTAAACCTTTTTCCCTCCCTTTTTTAAAAATTTTGCTACT
TGAAAACTTCTGATATATTTTAATTCCTTTT(poly)A

ty 1.1

VSG gene 1.1 sequence:

ATATACGACGGCAAAAGCGAGGAAATCAAACACAACAAGCAACGGCCGGT
TGCAACCAGCAAGGAGCTGATCAAATCGGTGTTGTACTACCGAAACAAGA
GACAGACAAATTTTTTACGCATTCAAACAGGCCTCGAGCAGACAAAAAGC
GACTCAACAAGGCGGAAACACCAAAATGCGAAGGAAGCAAAATCTGAATG
AGCCACCACTAAGGGTTGCGAATGGAATACGACAGAAGAAAGTGTAAACT
CACAGAAGACGCACAAAAGATGCAGAAAGCGAACACGAATAGGAAGCAGC
AACCAACCACAGGACACATTCTTTTGTCATTACAAAGGCCTTTGCTGCTT
TGCTGTTTATTTTACTTTTGTAGTTGCTCTGCTCTGTTTCCT(poly)A
STOP CODON

ty 1.a

VSG gene 1.A sequence:

GTCGACAGGAATTGAAAAAGCACTAGCAGAAGCGCAGACCACAAGCTACC
TCAAAGGCAAAATTGACGAATGGCTAAACATAGCGCACGAGACCAAGGGC

GCCTCGCACGGGTGCTTGACGGTCGCAGCGGGGACGACACGTCAAGAAGG
TCGGCAATATATCGACGGGCTCAAGTGCCCACTAACCCTCACAAAATTGG
CGAAAAAGCAAGGACACGAACAAGCTAACGGCCGGCGGATTTCAAGGCTC

AGGAGGCAGAGCGAGCAGCACACAGAGAGAGCCAT...AAGAAGCTAAAA
CAAGAAGAAGCTAACACAGACGAGAAGCACACTTGTTCGCTTAATCGCGC
GGCAGACAAGCCAAGGAAGGACAAGGCATTGAAACCTACAAAAGACAGCT

GTTCTCGACCACCCCTGGCAAGGAAATAGGCCGGCTAATGGCAAAAGTAG
CGACCTTAAGCTGCCACACAAATTTAGGAACCACCAAAGTCACGCCCCCC
CCCCCTGCAG

FIG. 9

tv 3 tail

3' hydrophobic tail
amino acid sequence:

ILTat 1.3	ser	ser	phe	ile	leu	asn	lys	gln	phe	ala	leu	ser	val	val	ser
1.11	ser	ser	ile	ser	leu	asn	lys	lys	phe	ala	leu	ser	met	val	ser
1.1	his	ser	phe	val	ile	thr	lys	ala	phe	---	---	---	---	---	---
221	ser	ser	ile	leu	val	thr	lys	lys	phe	ala	leu	thr	val	val	ser

ILTat 1.3	ala	ala	phe	ala	ala	leu	leu	phe	TAA
1.11	ala	ala	phe	val	ala	leu	pro	phe	TGA
1.1	ala	ala	leu	leu	phe	leu	leu	phe	TAG
221	ala	ala	phe	val	ala	leu	leu	phe	TAA

nucleotide sequence: 3' untranslated

```
221          TAA-------TTTCCCCCCTC------AAA-TTTCCCCCCTCC-TTTT---AAAATT
1.11         TGAAACAACATTTTCCCCCTCTTTTTTAAACCTTTTTCCCTCCCTTTTTTAAAAATT
1.1          TAG-----------------------------------------------------
             STOP            CCTC                CCTC          AmTn

221          TTCCTTGCTACTTGAAAACTTTTTGATATATTTTAACACCAAAACC(An)
1.11         ----TTGCTACTTGAAAACTT-CTGATATATTTTAATTCCTTTT(An)
1.1          ----TTGCT-CT-G----CT--CTG-T---------TTCCT(An)
                 octamer              14-mer
                                      11/1 match
```

- = no nucleotide

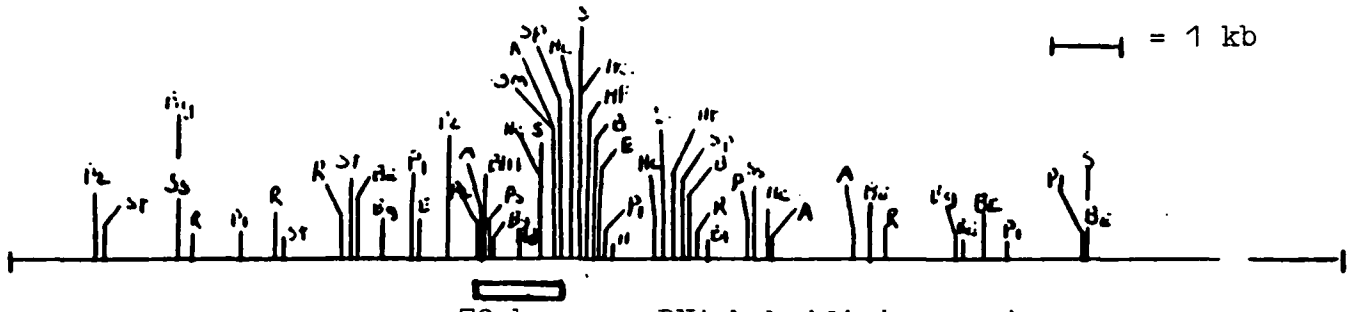
VAT	VSG genes in non expressor	Additional VSG gene in expressor	Expression mechanism
A	1 NT*, 1 T	- OR 1 T^+	ELC, NDA, TGC
1 (D)	1 T^+	-	NDA
3	6-8 NT**, 1 T (1LT)	1 T^+	ELC (TGC?)
5	2 NT**, 1 T	1 T^+	ELC
7	1 NT (*)	1 T^+	ELC
11	3 NT (*)	1 T^+	ELC

Table 4. Numbers of telomeric (T) and non-telomeric (NT) genes in IsTaR 1 VATs. Cloned non-telomeric genes in lambda 1059 = * and cloned expressed genes as cDNAs = +. Expression linked copy (ELC), Non duplication Activated (NDA) and Telomeric Gene conversion (TGC) mechanisms employed by each VAT are indicated. LT = Lingering telomeric ELC.

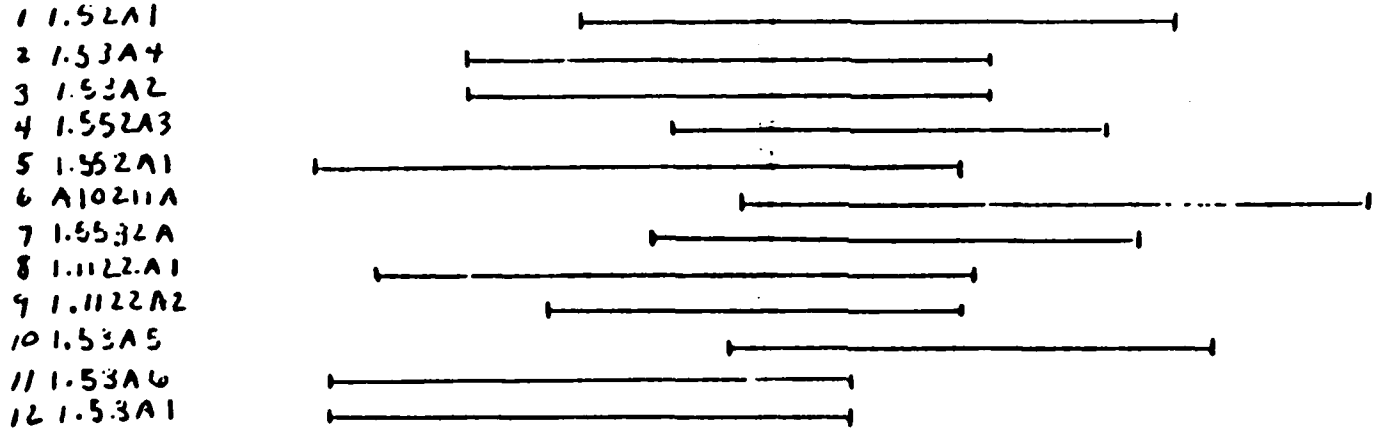
FIG. 10A
1 of 5

1.A gLNA MAP

= 1 kb

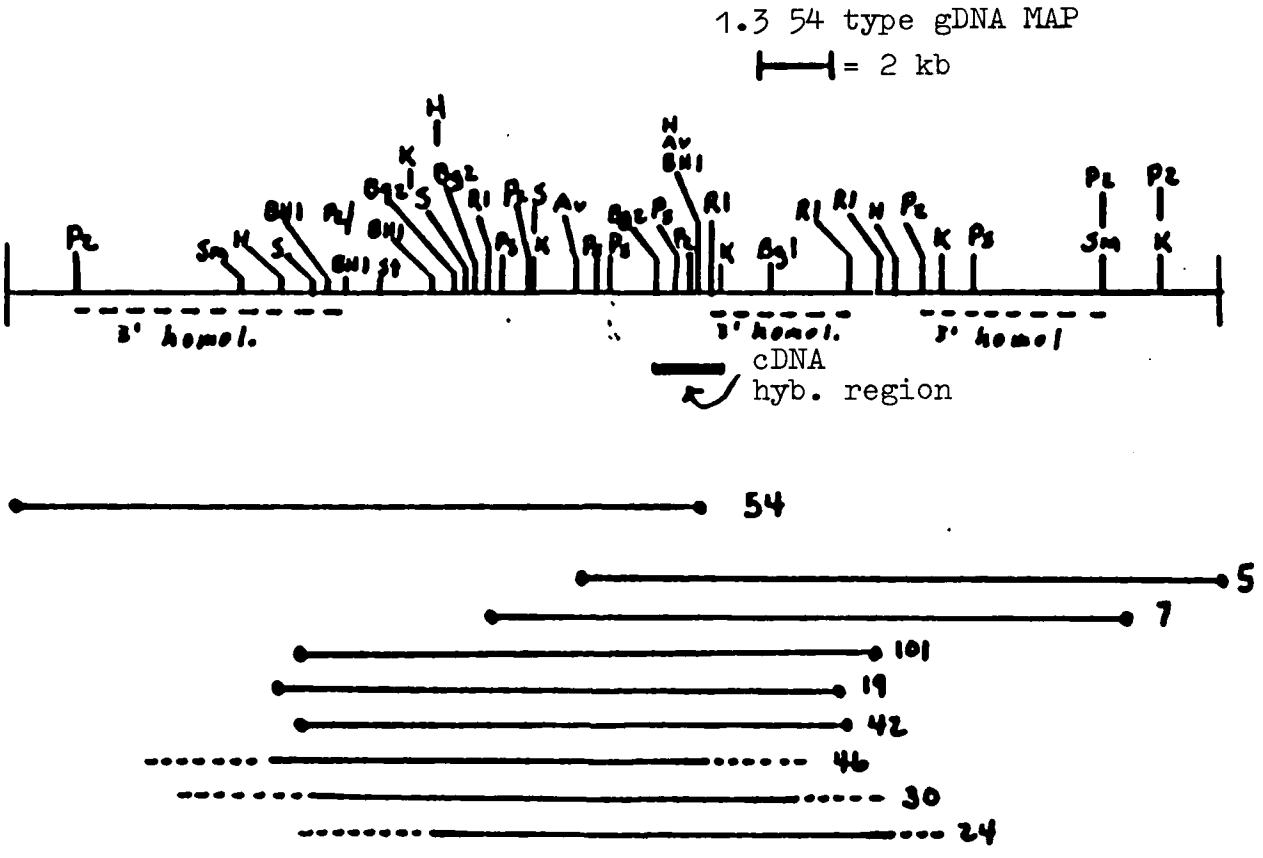


70 bp ◄ cDNA hybridizing region



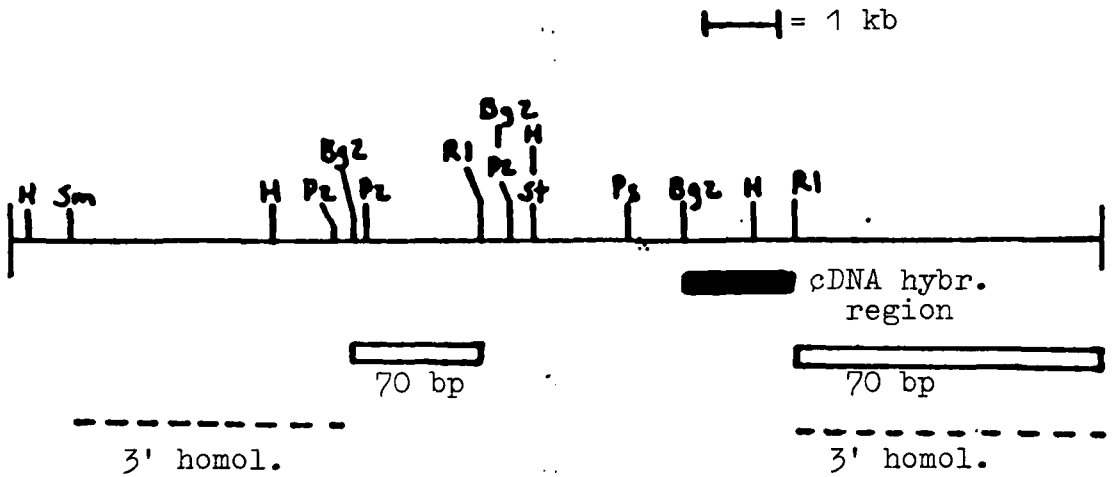
Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

FIG. 10B
2 of 5



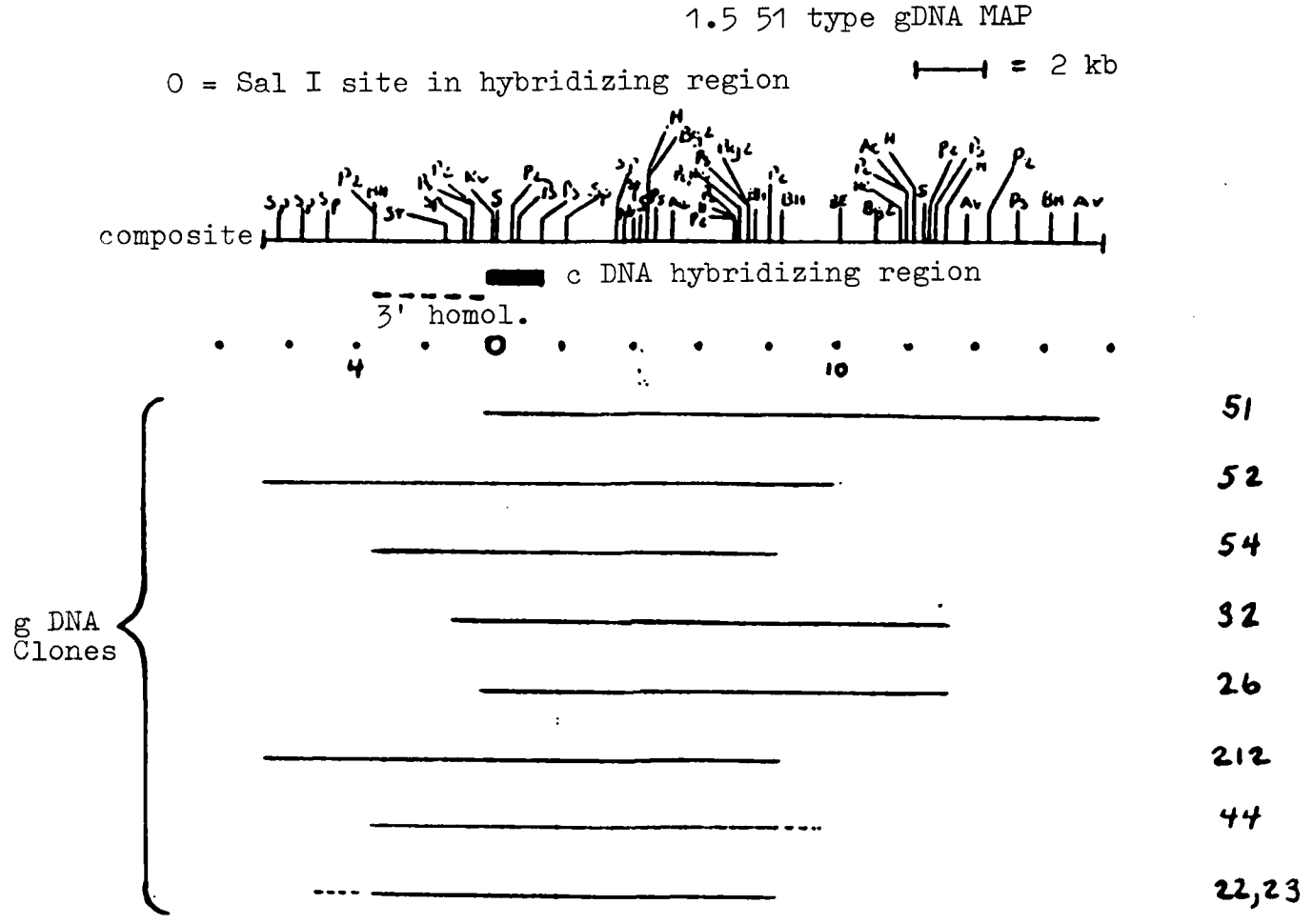
Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

1.3a type 2 gDNA MAP



Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

FIG. 10D
4 of 5



Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

1.5 46 - type g DNA MAP

O = Bg2 site in hybridizing region

= 2 kb

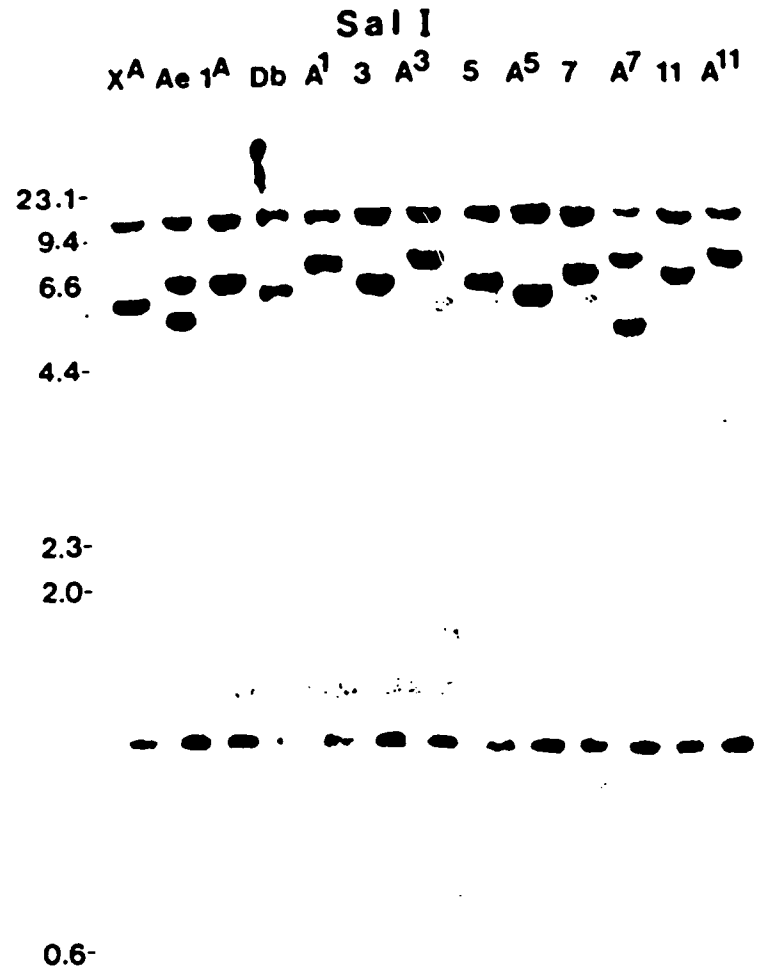
c DNA hybridizing region

g DNA Clone

46

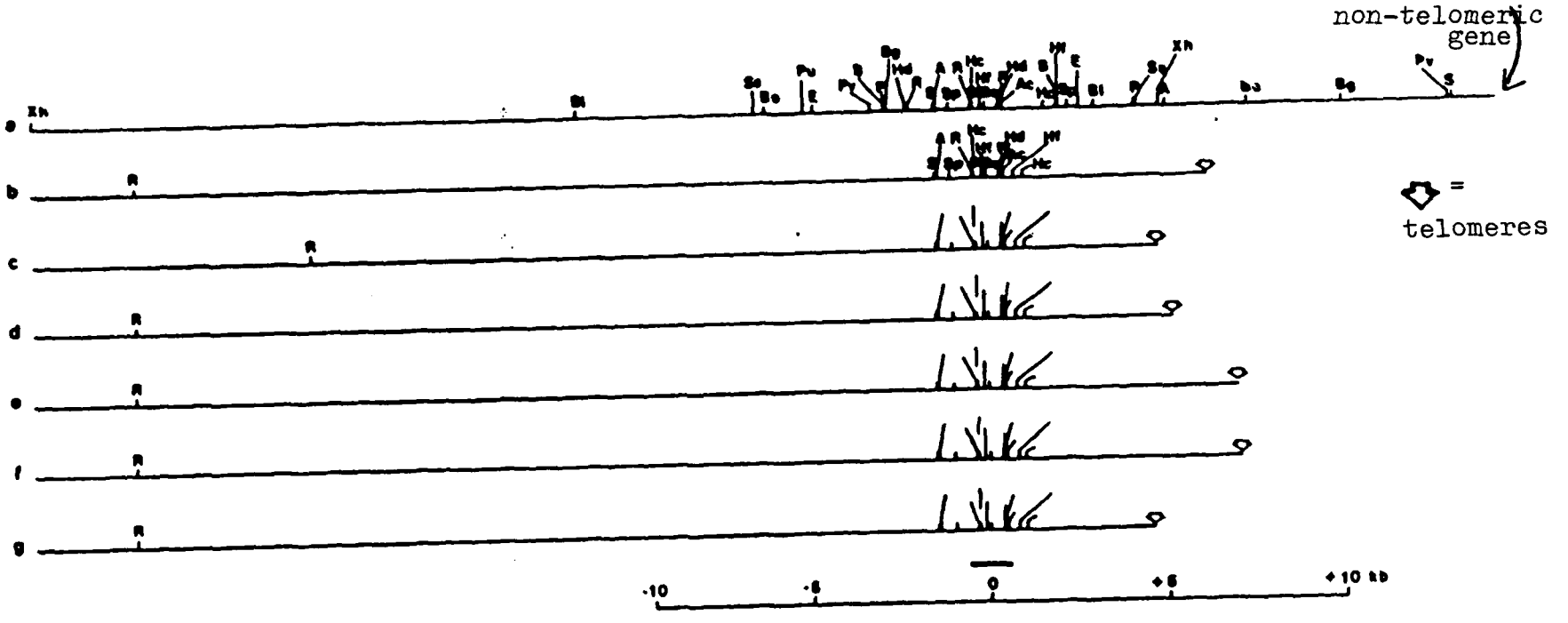
Ac=Acc I	BE=BstE II	Hc=Hinc II	Na=Nae I	S=Sal I
Al=Alu I	BN=BstN I	H=Hind III	Nr=Nar I	Sm=Sma I
Av=Ava I	CL=Cla I	Hh=Hha I	Pl=Pvu I	Sp=Sph I
BHI=BamHI	RI=EcoR	Hf=Hinf I	P2=Pvu II	St=Sst I
Bgl=Bgl I	RV=ECORV	Hp=Hpa II	Ps=Pst I	T=Taq I
Bg2=Bgl II	He=Hae III	K=Kpn I	R=Rsa I	Xo=Xho I

FIG. 11



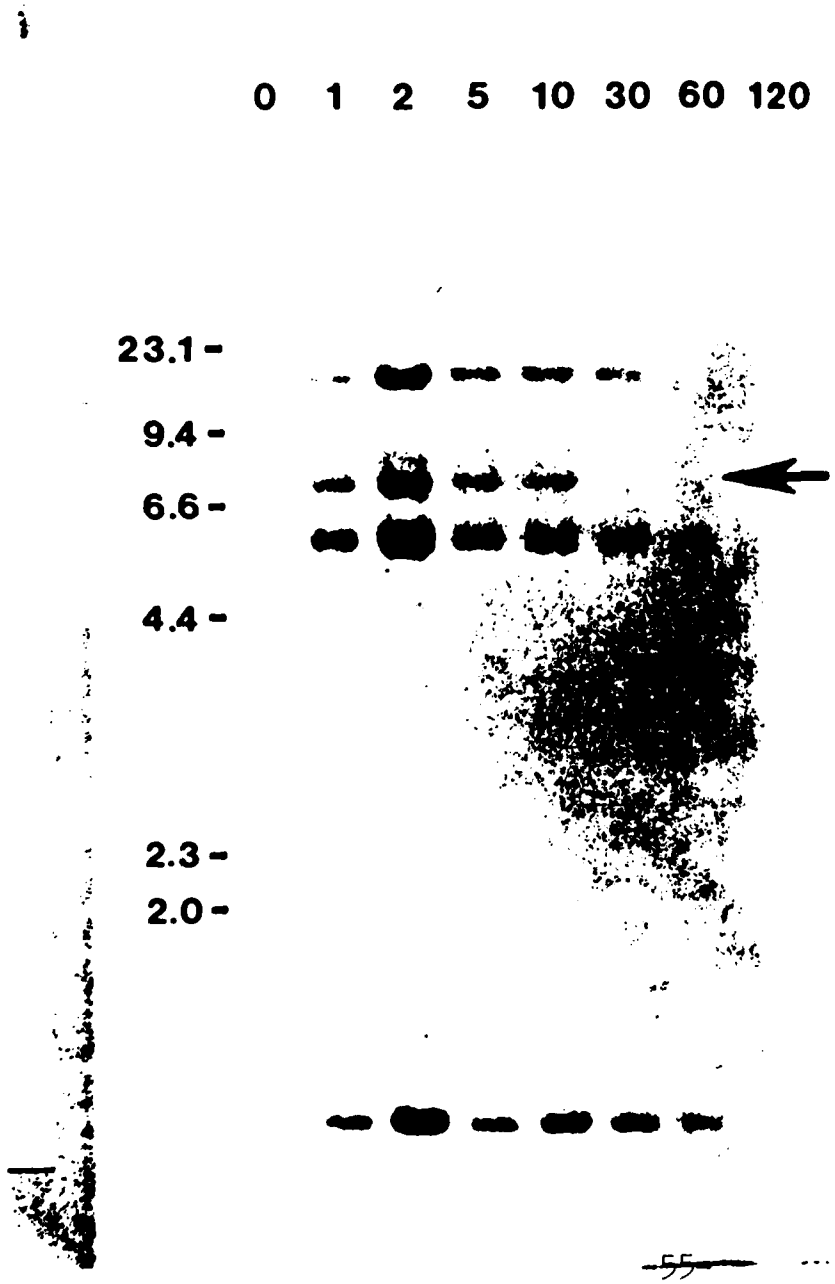
NOTE: 4 bands in Ae and A^7 but 3 in others

FIG. 12



VAT Ae = a+b+c
VAT A^1 = a+d
VAT ^1A = a+e
VAT A^7 = a+f+g

Figure 13



DNase I sensitive
(thus active) 1.A gene

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